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THE RECIPROCAL EFFECTS OF NITROGEN, PHOSPHORUS, AND POTASSIUM AS RELATED TO THE ABSORPTION OF THESE ELEMENTS BY PLANTS[1]

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INTRODUCTION

Importance of understanding energy relations between soil and plant

Coincident with the rapid advances in physical chemistry, concepts with respect to soil fertility problems are undergoing profound changes. This is manifested by the results of recent investigations (1, 5, 8, 9, 25, 28, 83, 95) in which the principles of plant production are shown to be dependent not only upon a knowledge of the exchanges of energy in the soil, but also upon the energy relations of chemical reactions between the soil and the plant, including necessarily the transformations of energy taking place within the living cells. This paper will treat of the factors affecting the equilibria of nitrogen, phosphorus, and potassium in the soil in relation to the absorption of these elements by the plant.

A mathematical expression for evaluating the factors of fertility

It will simplify our presentation of the subject of the so-called "selective" absorption of nutrient elements from the soil if we can find an expression that will enable us to visualize the dominant soil factors affecting the nutrition of plants (35).

Influence of water.—Water is without question the greatest limiting factor in plant development. It is known from numerous experiments, especially from those of Hellriegel (19) and of Wollny (94), that for maximum growth there exists for each soil type an optimum water content. If Hellriegel's experimental results (19) on the variation of dry matter produced as a function of the water content of the soil are plotted, it is found that the points lie approximately on a parabola. Let A (fig. 1) represent the yield when the water content of the soil is optimum and let Y represent the actual yield. Since the curve obtained is a parabola,

$$\therefore k(x_1 - x_0)^2 = A - Y \ldots\ldots\ldots\ldots\ldots\ldots (A)$$

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[1] Presented before the soil fertility conference held in commemoration of the 50th Anniversary of the Old Fertility Plots of the Pennsylvania State College. Publication authorized by the director of the Pennsylvania Agricultural Experiment Station as technical paper no. 534.

i.e., the decrease in yield from the maximum is proportional to the deviation in the water content of the soil from the optimum, or

$$Y = A - k(x_1 - x_0)^2 \qquad (B)$$

where k is a constant expressing the sensitiveness of the plant to changes in the water content of the soil.

Water content of the soil in relation to the maximum efficiency of mineral nutrients.—But Wollny's experiments (93) show that the optimum water content of the soil corresponds also to the maximum efficiency of the mineral nutrients, i.e., to their maximum absorption. Since growth from balanced nutrient solutions is a function of the amount of the nutrient element absorbed, therefore, the absorption of a nutrient element is also proportional to

$$A - k(x_1 - x_0)^2 \qquad (C)$$

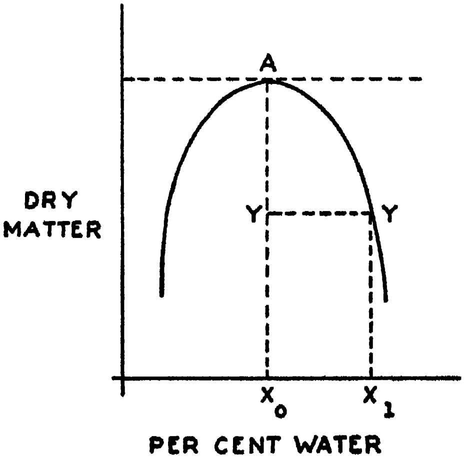


FIG. 1. RELATION OF YIELD TO WATER CONTENT OF SOIL

Let S_t represent the quantity of soil explored by the roots within a period of time t. Also let $\frac{1}{s}$ represent that fraction of the soil mass considered (S_t) entering into solution per unit of time in the form of a specific nutrient element (nitrogen, phosphorus, or potassium); and let $\frac{1}{r}$ be the fraction absorbed per unit of time by the plant. Then in a unit of time the fraction of the soil mass absorbed by the plant will be $\frac{S_t}{sr}$, where s measures the resistance to solution and r measures the physiological resistance of the roots to the absorption of the dissolved element (osmosis). The former can vary from $+\infty$ to 1. It is always great and depends on the nature of the soil. The latter can vary from $+\infty$ to 1 and depends on the feeding power of the plant (84, 89, 90, 91).

From (*C*) the absorption per unit of time is proportional to

$$A - k(x_1 - x_0)^2 \qquad \text{.............................} (D)$$

Let us put

$$\frac{1}{sr} = K\left\{A - k(x_1 - x_0)^2\right\}\cdot\frac{1}{s_1r_1} \qquad \text{.......... ....} (E)$$

The expression on the right represents then the fraction of the soil that passes from the solid condition to the plant in a unit of time.

Then, as we increase the time from *t* to *t* + *dt*, the amount absorbed (*dy*) will be

$$dy = K\left\{A - k(v_1 - x_0)^2\right\}\cdot\frac{S_t}{s_1r_1}\cdot dt \qquad \text{....................} (F)$$

where *K* is a constant representing the capacity of the plant. Or for the rate of absorption

$$\frac{dy}{dt} = K\left\{A - k(x_1 - x_0)^2\right\}\cdot\frac{S_t}{s_1r_1} \qquad \text{.......... ... .} (G)$$

In experimental work, of course, (*dt*) is relatively large; but it is remarkable that the average rate of absorption remains relatively constant for long periods of time (15 days, for example).

The equation shows that a soil can be deficient with respect to a given nutrient element for two reasons: (a) Inability, during periods of very active absorption, to maintain the supply above critical concentrations of the element. The function of fertilizers is to create a potential supply to provide for these emergencies. (b) Inability to provide at any time the supply of an essential element above critical concentrations. In this category are worn out or run down soils.

The equation modified by the addition of fertilizers.— Similarly for the rate of absorption of an element introduced in a soluble form such as a fertilizer which modifies a mass (say *S'*) of the soil, it may be shown that the rate of absorption of the element introduced is expressed by the equation

$$\frac{dy}{dt} = K'\left\{A' - k'(x_0 - x_1)^2\right\}\cdot\frac{S'_t}{s_1'r_1'} \qquad \text{.....................} (H)$$

The complete equation expressing the influence of the soil and of the added fertilizer is, therefore,

$$\frac{dy}{dt} = \underbrace{K\left\{A - k(x_0 - x_1)^2\right\}\cdot\frac{S_t}{s_1r_1}}_{\text{(fertility factor)}} + \underbrace{K'\left\{A' - k'(x_0 - x_1)^2\right\}\cdot\frac{S'_t}{s_1'r_1'}}_{\text{(fertilization factor)}} \qquad \text{..... ...} (I)$$

The formula clearly indicates the dominent rôle exercised by a soluble fertilizer in plant nutrition. As we shall see later the reciprocal influence of one element (ion) on another will involve the terms *s* and *r* of this equation.

The earliest experiments on the reciprocal effect of elements on absorption by plants

The reciprocal or mutual effect of one element upon another was first observed about the middle of the last century by Wolff (92). In his extensive nutrient solution culture investigations on the growth of barley, Wolff observed that the yield of plants grown in his "complete" nutrient solution was greater than that from cultures in which sodium was omitted. But Wolff's nutrient solutions were too high in potassium to be well balanced with respect to the concentration of this element. Analyses of the plants showed that the introduction of the sodium ion—from sodium chloride—decreased the absorption of potassium and so prevented its luxury consumption ("luxuskonsumption"), an expression which we have heard much about recently. Thus was it learned that absorption of an element in excess of that utilized might reduce yields and that this absorption in excess of the amount of the element required for the production of a normal plant could be controlled by the addition of another element—in Wolff's experiments, sodium. These experiments of Wolff's with sodium and potassium were confirmed by a number of investigators and definitely established the reciprocal action of at least two elements.

The concept of physiological balance

The existence of antagonism between other elements.—This reciprocal action of one element resulting in suppressing the absorption of another was later found to occur between other elements (ions) in the case of absorption by both plant and animal cells. A reciprocal relationship was found to exist between Ca^{++} and K^+ (10, 22, 27, 56), between NH_4^+ and K^+ (48, 49, 50), between Sr^{++} and K^+ (55), between Ca^{++} and Mg^{++} (51), between Na^+ and NH_4^+ (65, 66, 67), between SiO_3^{--} and PO_4^{---} (5, 16, 18, 43, 44), between Mg^{++} and Sr^{++} (55), between Mg^{++} and Ba^{++} (55), between Na^+ and Ca^{++}, between Na^+ and Mg^{++} (22, 27) and between K^+ and Fe^{+++} (15, 29).

Diminution of antagonistic action with dilution.—Loeb (49) gave the term "antagonism" to this phenomenon. But Osterhout (68) showed later that the antagonistic action increased with the increase in the concentration of the solution. In very dilute solutions, such as that of the order of magnitude obtained from soils by the various displacement methods, no antagonistic effect was observed. It follows that if the same mechanism is operative in soils as in nutrient culture solutions, it must be postulated that plant roots obtain their nutrients from a much more concentrated solution than that obtained thus far by artificial means.

Optimum ratios.—These investigations by Loeb and Osterhout led to the development of the concept of physiologically balanced solutions, from which followed the long search by means of nutrient culture solutions to discover the best (optimum) ratios between the various nutritive elements (7, 20, 32, 47, 52, 53, 54, 73, 74, 76, 77, 78, 79, 88).

Nutrient culture versus field experiments

Absorption from soil and nutrient solutions compared.—The principles acquired by means of these laboratory studies cannot be overestimated with respect to the service rendered in providing a system of physiological knowledge for application to field studies. The environmental conditions, however, affecting the absorption of elements from the soil are different from those under which nutrient culture experiments are conducted because: (a) The rate of diffusion of ions toward the root zones is more rapid in nutrient culture solutions than in the soil (21); (b) the extent of the root system and, therefore, the absorbing surface is greater under field conditions than in nutrient culture solutions. The factor $\frac{1}{s_1 r_1}$ of equation (*I*) thus becomes unity in solution but not in soil cultures.

Soil versus plant analysis.—Because the concentration of the soil solution with respect to any of the principal elements is subject to wide fluctuations even in the uncropped condition (2, 3, 80), it is difficult to draw conclusions relative to absorption of ions from soil solution studies alone. It is possible, however, as we shall see by comparing the amounts of nutrients absorbed—at the different vegetative periods—by plants growing under uniform soil and meteorological conditions, to extend very considerably our knowledge of the factors influencing the absorption of the principal (dominant) elements (ions) from soils.

THE LAW OF THE MINIMUM AND ITS IMPLICATION

Many "laws" have been advanced purposing to define the relationship between the amount of nutrients absorbed by the plant and the dry matter produced (45, 46, 60). The first was by Liebig (45), who announced as a corollary to what afterwards became known as the "law of the minimum" that nutrient elements must be absorbed in certain definite proportions and in such a manner that when the rate of supply of one of the principal nutrient elements is reduced below the critical concentration for that element the rate of absorption of the other principal nutrient elements is retarded or depressed, because an inadequate supply will render the other elements unavailable, since the plant will be unable to grow to any extent. Consequently, assuming all the other growth factors constant, it follows from Liebig's basic hypothesis that if, with time as the abscissa and the quantity of nutrient element absorbed during the growth of a plant as ordinate, graphs are plotted indicating the course of absorption of nitrogen, phosphorus, and potassium from a soil to which a "complete" fertilizer has been added—with all other growth factors constant—the graph showing the course of absorption of any one of these elements from a fertilizer containing only two of them would be displaced from its former position and would now fall belo

The results of field experiments

The American field experiments.—The results which we have obtained in our experiments on the absorption of these elements by apple trees grown in the field under controlled conditions (86) are shown graphically in figure 2. The data are expressed in percentage of the element absorbed by the last season's branch growth on a moisture-free basis. These results are in accordance with Liebig's basic hypothesis. Thus, it will be observed that the omission of any one of the elements—nitrogen, phosphorus, or potassium—from the fertilizer has resulted in a decreased absorption of the remaining elements and is accompanied by decreased growth and reproduction. Analytical data recently obtained with respect to the total amount of each element taken up by an entire tree show the same relationships.

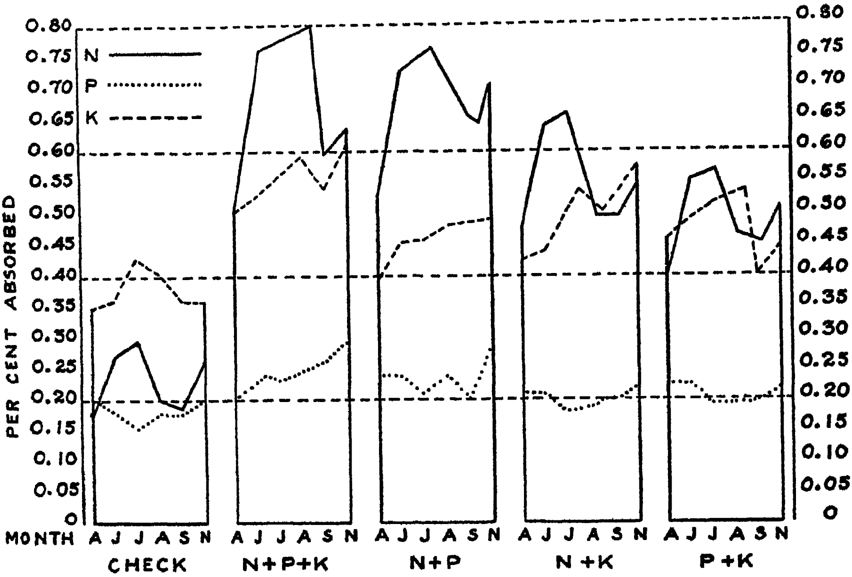


FIG. 2. THE COURSE OF THE ABSORPTION OF NITROGEN, PHOSPHORUS, AND POTASSIUM BY *Pyrus malus L.* AS DETERMINED BY THE PERIODIC ANALYSIS OF THE CURRENT AND SEASON'S BRANCH GROWTH

Results are expressed in percentages of dry weight of material

The French field experiments.—But the results of very carefully conducted long time experiments by Lagatu and Maume (34, 36, 37, 38, 40) in Montpelier on the course of absorption of nitrogen, phosphorus, and potassium by the vine (*Vitis vinifera*) show that in wet as well as dry years the omission of one of the three elements—nitrogen, phosphorus, potassium—from the fertilizer resulted not in a decreased absorption of the remaining elements relative to that from the complete fertilizer, as would be expected from Liebig's law, but on the contrary in decreased yields produced by a nutritional lack of balance caused by an *increase* in the absorption of the remaining elements of the fertilizer. The results shown in figure 3 are given in percentages of the dry weights of leaves.

These results are of paramount importance in the science of plant and animal nutrition and in their application to the problem of the fertilizer requirements of soils. They show the impossibility of making a comparison between the composition of plants of the same species unless the data are referred to the same standard control, i.e., to the composition of the plant of the same species grown under such conditions of physiological balance as to produce sufficient growth to ensure reproduction of the highest order.

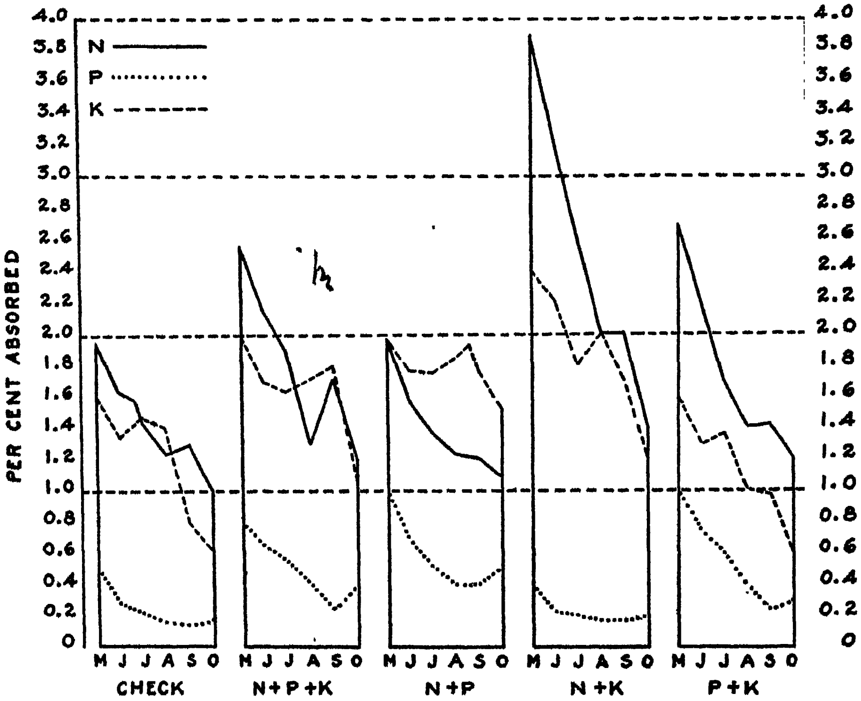


FIG. 3. THE COURSE OF THE ABSORPTION OF NITROGEN, PHOSPHORUS, AND POTASSIUM BY *Vinis vitifera* AS DETERMINED BY THE PERIODIC ANALYSIS OF THE LEAVES

Results are expressed in percentages of dry weight of material

It is important to ascertain the causes of these apparently anomalous results. But before attempting an explanation the results of some experiments in Austria on barley (75) bearing on this same problem will be given.

The Austrian field experiments.—Both nitrogen and phosphorus in the soil of this experiment are very low but the potassium content is high. The results, which are given in absolute amounts of the element absorbed (fig. 4), show that relative to the absorption of elements from the (N + P + K) plot the omission of potassium resulted in an increased absorption of phosphorus and nitrogen and that relative to the (2N + P + K) plot the reduction in nitrogen (N + P + K) resulted in a decreased absorption of potassium and phosphorus.

In the unfertilized plot potassium, as would be expected from Liebig's law, is absorbed in relatively greater amounts than nitrogen or phosphorus up to the time of blossoming. The analyses of his plants, however, showed that after this period potassium migrated again to the soil. Sekera maintains that this migration is a physiological necessity and represents the plant's attempt to rid itself of an element absorbed in excess of that utilized.

It will be observed that when nitrogen and phosphorus are added (N + P) the absorption of the elements is increased and potassium does not migrate back to the soil, for the excess of potassium is now balanced by the increased absorption of nitrogen and phosphorus. The addition of potassium to this soil already abundant in this element, as will be seen from the (N + P + K) plot, causes a migration of potassium to the soil and a decrease in the amount of nitrogen and phosphorus absorbed. On the further addition of nitrogen, the absorption (2N + P + K plot) again becomes balanced and results in a maximum yield. Further additions of nitrogen result in an excess absorption of this element with migration to the soil and a decreased absorption of potassium and no influence on the absorption of phosphorus.

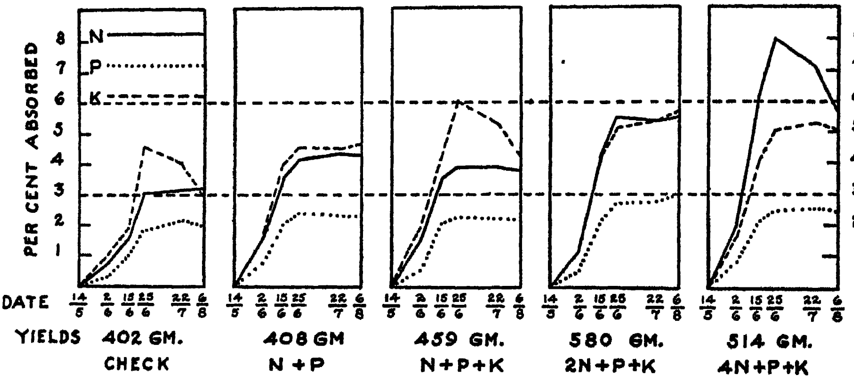


FIG. 4. THE COURSE OF THE ABSORPTION OF NITROGEN, PHOSPHORUS, AND POTASSIUM BY *Hordeum sativum* AS DETERMINED BY THE PERIODIC ANALYSIS OF WHOLE PLANTS

Results are expressed in percentages of dry weight of material

An analysis and synthesis of the results

Lagatu and Maume (35, 39, 40, 41, 42) have announced that their results clearly show that the corollary from Liebig's "law of the minimum" is not capable of generalization. The present writer will show, however, that the contradiction is an apparent and not a real one.

It may be seen from equation (I) that the effects of the addition of nitrogen, phosphorus, or potassium to a soil, added singly or in one or more combinations, will—all other factors remaining constant—depend upon the factor $\left(\frac{1}{s_1} + \frac{1}{s_1}\right)$, i.e., (a) upon the supplying power of the soil to maintain concentrations of an element above the critical concentration, and (b) to the concentration with respect to that element produced by the added fertilizer. A knowledge of this supplying power with respect to a given nutrient element would, therefore, appear to be essential in order to determine the ratio between the constituents of a fertilizer necessary to provide a balanced soil solution. Unfortunately, the process of obtaining a true measure of the supplying power with respect to a given nutrient ion is laborious, involving long and expensive experiments (80).

For the purpose of the present analyses, however, sufficient information may be obtained from laboratory methods similar to those of König (33), and of Neubauer (61, 62, 63, 64, 87) and to the displacement (31, 69, 72) or extraction methods (2, 3). Information of this type is available (39, 40, 41, 42, 75, 85) for the check plots of the three experiments discussed. This information is assembled in table 1.

TABLE 1

Amounts of total solutes and of "available" nitrogen, phosphoric acid, and potash in the soils from the three experiments considered

LOCATION OF EXPERIMENT	DRY SOIL			
	Total solutes	Soluble in 1 per cent K_2SO_4	Soluble in 1 per cent citric acid	
		N	P_2O_5	K_2O
	p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*
State College, Penna, U. S. A.	310–490	82	65	130
Montpellier, France	2,000–2700	260	1,300	210
Vienna, Austria	......	(low)	47	280

It will be observed that when the soil is deficient in an element, i.e., when s_1 of equation (I) is high $\left(\text{or when } \frac{1}{s_1} \text{ is small}\right)$, the concentration being below the critical level for that element (e.g., nitrogen and phosphorus in the Pennsylvania and Austrian experiments), the omission of the deficient element from the fertilizer has resulted in a *decreased* absorption by the plant of the remaining elements contained in the fertilizer added. Under these conditions the omission of the element from the fertilizer must result in a greater relative deficiency of this element compared with the other two elements and, accordingly, limits (decreases) their absorption as predicated by Liebig's "law."

But when an element is relatively abundant, i.e., when s_1 of equation (*I*) is small, or when $\frac{1}{s_1}$ is large [e.g., nitrogen and phosphorus in the French experiments (34, 36, 37, 38, 39, 40, 41, 42) and potassium in the Austrian experiments (75)], the omission of that element from the fertilizer would not reduce $\frac{1}{s_i}$ of equation (I) below the critical concentration for that element. Consequently, the omission of an element already abundant in the soil from a fertilizer results in an *increased* absorption by the plant of the remaining elements added in the fertilizer *relative* to the absorption of these elements from the complete fertilizer plot until the element omitted from the fertilizer becomes a limiting factor, i.e., until its concentration is reduced below the limiting concentration. *The whole problem, therefore, is one of relativity.* In no case is Liebig's law violated.

It should now be apparent that all statements in the literature bearing on the seemingly contradictory results of one worker with those of another on the problem of the mutual action of one element on the solubility of another and of its absorption by plants can be satisfactorily explained in the light of the foregoing interpretation.

The so-called "luxury" consumption of elements

All the experimental evidence (75) appears to indicate that the plant, especially in the early stages of growth up to blossoming, absorbs the nutrient ions in approximately the same proportion that these ions are present in the nutrient medium. Accordingly, if $\frac{1}{s_i}$ or $\left(\frac{1}{s_i} + \frac{1}{s_i'}\right)$ of equation (I) is so great as to be out of physiological balance for any element, a "luxuskonsumption" (93) will occur. And, as we have seen, such "luxury consumption" may result in a disturbance to normal metabolism sufficient to affect growth and reproduction (34, 36, 37, 38, 39, 40, 41).

Sekera's experiments (4, 75) which give clear evidence that the excess of an element above that which the plant utilizes in metabolism may be returned to the soil shortly after blossoming occurs have already been discussed. Ions, therefore, may be expelled from the plant in the process of adjustment to more favorable equilibrium conditions. Hoagland's experiments (22, 27) would lead to the view that the mechanism *of this expulsion may be one of exchange of anions or of cations.*

Sekera's experiments (75), moreover, show that within the limits set by the amount of water and solar energy available the return of an element absorbed in excess of that required may be prevented by balancing it through the addition of the other two principal (dominant) nutrient elements (ions), thus enabling the plant to utilize this excess (fig. 4). These experiments, moreover, are of additional interest in indicating a method by means of which the required physiological balance between nitrogen, phosphorus, and potassium may be obtained.

No "luxury" consumption indicated in the old fertility plots of the Pennsylvania State College.—It is of interest to note that results obtained on the absorption of nitrogen, phosphorus, and potassium by wheat in the old fertility plots having the highest yields of the Pennsylvania State College (56) do not indicate any absorption of an element in excess of that utilized. A comparison of the amounts of these elements absorbed by wheat on plots 17, 19, and 21, receiving increasing amounts of nitrogen as dried blood, shows that the highest application of nitrogen (72 pounds) has not resulted in a lack of balance. The results are given in table 2. We may deduce from this that yields might be further increased by increasing the rate of application of one or more of the carriers of the nutritive elements used in these plots.

TABLE 2

Amounts in pounds per acre applied and recovered in entire wheat crops, of nitrogen, potash, and phosphoric acid on dry basis (after MacIntire)

PLAT NUMBER	TREATMENT	NITROGEN		POTASH		PHOSPHORIC ACID	
		Applied to soil	Removed by plant	Applied to soil	Removed by plant	Applied to soil	Removed by plant
16	6 tons manure	59	34 8	52	37.6	38	18 5
17	(1N)PK	24	29.3	100	42.0	48	17 6
18	8 tons manure	78	39.2	69	36.5	51	19.8
19	(2N)PK	48	37.7	100	44.6	48	20.0
20	10 tons manure	98	44.4	86	46.0	64	22 2
21	(3N)PK	72	45.3	100	61 7	48	24.2
22	6 tons manure + CaO	..	36.4	...	34.9	..	21.6
24	Check	..	18.4	...	16.1	..	9.3

Definition of an optimum fertilizer ratio

Physiological balance within the plant once obtained may again be easily disturbed by the addition of any one of the principal nutrient elements to the soil. *The optimum ratio of the constituents of a fertilizer may then be defined as that ratio which, on the addition of any one of its nutrient constituents—nitrogen, phosphorus, or potassium—results in no increased utilization by the plant of any of the other elements as determined by the time-absorption-graph method.*

The extension of the concept of ionic balance to all elements

The influence of calcium ions.—For the purpose of simplification, the effect of the reciprocal action of elements on absorption has been confined in the present discussion to the three principal nutrients—nitrogen, phosphorus, and potassium—because under the conditions commonly encountered these three elements are the dominant and, indeed, controlling regulatory factors in plant metabolism (71), the functions of which cannot be replaced by any other element. Nevertheless, in practice it is necessary to take cognizance of the effect of other elements and especially of calcium when applications of lime are made regularly. The effect of calcium oxide (or carbonate) on the solubility of soil nitrogen, phosphorus, and potassium will depend upon the physical and chemical state in which they are present. On the old fertility plots of the Pennsylvania State College CaO and $CaCO_3$ have resulted in depressing the solubility of soil potassium (2, 3). Moreover, this depression in *solubility* is associated, as would be anticipated, with a decreased *absorption* of potassium by the plant (56).

THE MECHANISM OF THE RECIPROCAL ACTION OF IONS IN THEIR RELATION TO ABSORPTION

The nature of the functioning mechanism has not as yet been elucidated. The amount of an ion absorbed in unit time is expressed by the fraction $\frac{1}{sr}$ of equation (E), i.e., by the product of the solubility and osmotic value of the respective ions. It is dependent, therefore, upon the structure, chemical composition, and pore space of the root-absorbing surfaces on the one hand, and upon the degree of hydration, atomic volume, and the magnitude of the dissociation constant of the ions, on the other (17, 24, 57, 58, 59, 81, 82).

The relation of dissociation constants to antagonism

The significance of this latter influence is indicated by the experiments of Maume (57, 58, 59) on ion antagonism. In the case of binary electrolytes antagonism is related to the conductivity of the salts after mixture. The antagonistic effect is greatest at that particular concentration of ions represented by the intersection of the dissociation curves of each salt separately determined at dilutions corresponding to the proportions at which each salt is present in the mixture and which is indicated on the abscissa SS' (fig. 5). Thus, in the case of NaCl and $CaCl_2$, of all possible proportions of 0.12 Mol. NaCl (soln. S) and 0.164 Mol. $CaCl_2$ (soln. S_1) the least toxic mixture is at the point A of intersection of the dissociation curves represented by a mixture of 5 cc. $CaCl_2$ + 95 cc. NaCl.

Antagonism is positive.—In the case of binary mixtures of alkali and alkaline earth salts having a common anion the maximum antagonism occurs when the quantity of each salt is such that if separately dissolved in the same volume of water as that of the mixture this would give the same coefficient of ionization. This is indicated by the point represented by the intersection of the curves in figure 5. In this case the common anions will be at different concentrations. For example the molar concentration for NaCl, $CaCl_2$, $Ca(NO_3)_2$, and $NaNO_3$ having the same conductivity are 0.12, 0.22, 0.086, and 0.029, respectively.

Antagonism is nil.—The antagonism is *nil* when, as in the case of the chlorides of the monovalent ions Na^+, K^+ and NH_4^+, for example, the dissociation constant of the mixture is approximately unaltered. This holds when salts having a common ion have, at equal molar concentrations, the same value for the dissociation constant.

Antagonism is negative.—The antagonism is *negative* when, as the result of double decomposition, the degree of dissociation is increased by the formation of different kinds of molecules and ions. Thus:

$$AB + CD = AB + CD + AD + CD + A^+ + B^+ + C^+ + D^+. \qquad .(J)$$

Causal factors as yet undetermined

These experiments of Maume are of interest in pointing the way to an explanation of the mechanism of antagonism between ions of the *same* electrostatic charge. Nevertheless, because of the present transition stage of our knowledge concerning the dissociation of strong electrolytes, it would appear that the dissociation constant is only a measure of a causal factor that is as yet obscure. The influence of the motility of ions in relation to dissociation must be considered in determining the actual causal factors operative.

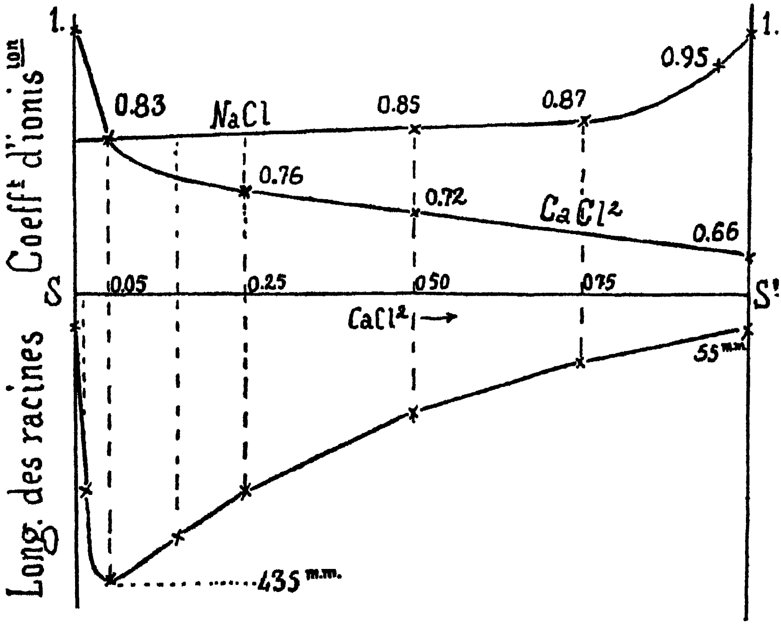


Fig. 5. Relation of the Length of the Roots of Wheat Seedlings Growing in Solutions of 0.12 Mol. NaCl (Solution S) and of 0.164 Mol. $CaCl_2$ (Solution S_1) in the Proportions Indicated to the Dissociation Curves of the Separate Salts Determined at Dilutions Corresponding to the Proportions at Which Each Salt is Present in the Mixture

THE INFLUENCE OF OTHER FACTORS IN PRODUCING SELECTIVE ABSORPTION BY PLANTS

The absorption of the so-called physiologically acid and alkali salts

The relation of hydrogen-ion concentration to the absorption of the principal nutrient elements is gradually being elucidated by the application of the rapidly accumulating facts of physical chemistry. By such means it is possible not only to explain but also to predict the physiological effects of applications of salts such as sodium nitrate and ammonium sulfate.

The rate of absorption of ions under the conditions of hydrogen-ion concentration existent in normal soils (pH 5.5 — 7.0) follows the lyotropic series of Hofmeister (30), viz., for cations the series, in descending order of mobility, is $H > K > Na > Li > Mg > Ba > Sr > Ca$ and for anions $OH > \frac{Bi}{I} > NO_3 > Cl > HPO_4 > SO_4$ (17). But at hydrogen-ion concentrations outside the aforementioned range the series is irregular and non-predictable (17).

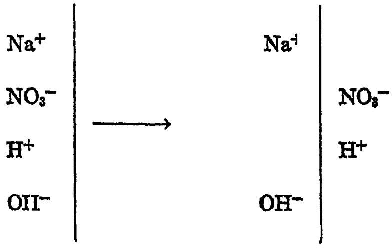
The effect of the hydrogen-ion concentration of the substrate.—A reciprocal action resulting in "selective" absorption may take place between a cation and an anion not only in the presence of a non-diffusible ion (11, 12, 13, 14) but also when no non-diffusible ions are present. If the substrate is slightly acid, neutral, or alkaline, the membrane (roots) will, as a result of the absorption of OH^- ions, become negatively charged. The electrostatic attraction resulting from this potential difference will result in a more rapid absorption (osmosis) of cations than of anions (28). Under these conditions the cation is dominant. But, on the other hand, in an acid medium (especially pronounced below certain critical values *circa* pH 5.5), the membrane (roots), as a result of greater absorption of H^+ ions, becomes positively charged and the electrostatic attraction resulting from this potential difference causes anions to be absorbed faster than cations (28). Under these conditions the anion is dominant. The results of the investigations of Hoagland (23, 26), of Butkewitsch (5, 6) and of Hager and Stollenwerk (17) on the absorption of ions from solutions of $NaNO_3$, $Ca(NO_3)_2$, NH_4OH, $(NH_4)_2SO_4$, and NH_4NO_3 may be interpreted in this manner. ***The relative effects, therefore, of these salts may be altered at will by changing the concentration of the hydrogen-ion.*** The process may be visualized as follows:

DEGREE OF ACIDITY	CHARGE ON ROOTS	SALT APPLIED
Slightly acid, neutral or alkaline.		$\underline{NH_4^+} > SO_4^{--}$ $-\underline{Na^+} > NO_3^-$
Distinctly acid, pH 5.5 and below.	+ +	$-\underline{SO_4^{--}} > NH_4^+$ $-NO_3^- > Na^+$

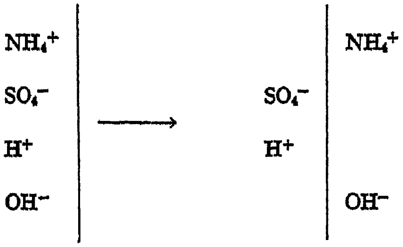
where underlining indicates the ion preferentially absorbed, and > indicates the rate of absorption is greater than.

The fact that in alkaline, neutral, or slightly acid media cations exert the dominant influence in absorption, and conversely that in acid media the anions are the dominant factor explains the superiority of ammonium sulfate over sodium nitrate under the former conditions and of sodium nitrate over ammonium sulfate under the latter. The physiological effect of ammonium salts will accordingly become relatively weaker as the H^+-ion concentration *increases*, whereas the physiological effect of sodium nitrate, on the other hand, will be weaker as the H^+-ion concentration of the soil *decreases*.

The influence of membrane hydrolysis on the "selective" absorption of ions.—It can be shown from thermodynamic principles that an ion cannot permeate the cell membrane without an oppositively charged ion (17). If a dialyzable electrolyte is present on one side of a semi-permeable membrane and on the other pure water, a hydrolytic decomposition of the salt occurs. Using Na^+ as the cation and NO_3^- the anion the process may be illustrated thus:



The median will, therefore, tend to become alkaline because of the much slower rate of penetration of Na^+ compared with NO_3^-. Na^+ is much more strongly hydrated than NO_3^-. On the other hand, if $(NH_4)_2SO_4$ is used the medium will tend to become acid because of the much greater rate of penetration of NH_4^+ compared with $SO_4^{=}$. $SO_4^{=}$ is more strongly hydrated than NH_4^+. Thus:



This hydrolytic action due to the presence of the membrane is promoted in accordance with the Gibbs-Donnan law by the difficultly permeable Na^+ ions in the one case and the SO_4^- ions in the other.

These interpretations are supported by the results of numerous experiments. For example, Prianishnikov (70) found that sugar beet and maize grown in sand cultures, with frequent renewal of the culture solutions so as to maintain practically constant reactions, gave much better results with ammonium salts than with nitrate salts at pH 7.0 but much poorer results with ammonium salts than with nitrate salts at pH 5.5. And Hager and Stollenwerk (17) found that the ratio of nitrogen to sulfate $\left(\frac{N}{SO_4}\right)$ absorbed from ammonium sulfate by rye, oats, wheat, and barley ranged from 3.06:1 for rye to 5.4:1 for oats.

On the old fertility plots of the Pennsylvania State College the response from ammonium sulfate was better than from sodium nitrate during the first decade but afterwards, as the hydrogen-ion concentration of the soil of the ammonium sulfate plots increased, the response from sodium nitrate was much better than from ammonium sulfate. The pH range of the ammonium sulfate plots is at present (tier I) 4.18–4.50 and of the sodium nitrate plots 5.51–5.71.

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STUDIES ON THE DISPERSION PROCEDURE USED IN THE HYDROMETER METHOD FOR MAKING MECHANICAL ANALYSIS OF SOILS[1]

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In previous communications the hydrometer method has been offered as very rapid, simple, and reasonably accurate for making mechanical analysis of soils. It has been shown (1, 2) that by this method the "total colloid" of soils can be determined in 15 minutes, the conventional clay (0.005–0.000 mm.) in 1 hour, and the fine clay (0.002–0.000 mm.) in 2 hours. In seeking an explanation for this extreme rapidity, which is contrary to Stokes' law, the question has arisen whether it may not be due to an incomplete dispersion of the soils, especially in view of the fact that the soils are dispersed for only about 10 minutes by the mechanical stirrer.

To answer this question certain special tests have been made; they are reported here.

DISPERSING SOILS WITH MECHANICAL STIRRER AND SHAKER

With the exception of the sands and the soils not easily dispersed, the hydrometer method calls for stirring the soils for about 10 minutes. The U. S. Bureau of Soils allows (3) the soils to be shaken for 16 hours for the purpose of mechanical analysis. Comparisons between mechanical analyses of soils which had been stirred 10 minutes with the mechanical stirrer and those of the same soils after 16 hours' shaking with the standard horizontal shaker are reported in table 1.

Table 1 shows clearly that stirring the soils 10 minutes with the mechanical stirrer produces about the same degree of dispersion as shaking them 16 hours with the shaker. With the exception of Fargo clay surface soil and some of the sandy soils, the differences are small and mainly within the limits of experimental error. The larger variation in the Fargo clay surface soil is probably due to the undecomposed organic matter, which on standing a longer time in water undergoes more dispersion in the shaker. The variations in the sands are due to the tendency of the mechanical stirrer to break down the sand particles on long stirring.

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TREATMENT WITH HYDROGEN PEROXIDE

The international pipette (3) method for making mechanical analyses of soils calls for treating them with hydrogen peroxide, whereas the hydrometer method has not called for such a treatment.

TABLE 1

Comparison of mechanical analyses of soils which were stirred 10 minutes with dispersing machine and those shaken 16 hours with shaker

	STIRRED 10 MINUTES			SHAKEN 16 HOURS		
	Colloid	Clay, 0.005–0.000 mm.	Fine clay, 0.002–0.000 mm.	Colloid	Clay, 0.005–0.000 mm.	Fine clay, 0.002–0.000 mm.
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Plainfield sand 0–6 inches	10.7	8.8	6.9	9.5	7.1	6.0
Plainfield sand 6–18 inches	9.6	7.0	5.5	8.4	6.0	4.6
Miles fine sandy loam	10.7	8.8	8.2	9.0	7.2	7.4
Kirven sandy loam	15.7	10.8	9.9	14.0	9.3	8.3
Beardon fine sandy loam, surface	19.6	16.8	13.7	21.6	19.7	17.6
Amarillo fine sandy loam	24.0	19.0	17.0	22.8	18.2	16.0
Loess soil 0–10 inches	25.6	14.5	12.9	26.4	13.8	12.5
Colby silt loam, surface	35.5	22.1	19.1	36.3	23.2	20.8
Colby silt loam, subsoil	35.0	25.5	19.0	36.2	26.0	22.1
Barnes loam	41.1	32.2	29.2	41.8	33.6	30.4
Cecil clay loam, subsoil, unlimed	42.2	37.7	36.5	41.8	38.1	35.3
Cecil clay loam, subsoil, limed	44.3	40.2	38.7	43.3	38.7	36.7
Ava silt loam	42.0	33.6	30.7	42.1	32.9	30.2
Grundy silt loam, subsoil	49.0	36.4	34.0	48.6	35.7	33.6
Lake Charles clay, surface	50.2	42.4	38.9	50.7	42.7	39.8
Lake Charles clay, subsoil	56.2	46.0	42.2	57.6	47.8	43.6
Aberdeen clay 4–10 inches	52.3	45.4	42.9	51.7	44.8	42.5
Welland clay, surface	59.8	49.5	42.5	60.0	51.2	43.1
Welland clay, subsoil	73.0	63.7	56.5	73.8	63.5	57.1
Fargo clay, surface	62.0	45.2	38.9	63.7	53.0	43.9
Fargo clay, subsoil	73.4	59.8	53.6	75.2	62.6	55.1
Osage clay	76.2	61.5	56.8	74.2	62.7	57.3
Parson silt loam B	75.0	64.3	60.1	74.7	65.1	59.2
Parson silt loam hardpan 24 inches	72.2	67.5	67.3	74.1	67.1	63.9
Kindersley clay 1–3 inches	77.6	75.9	72.2	76.9	74.4	72.8
Kindersley clay 3–8 inches	78.2	75.0	71.7	79.4	75.6	72.5
Sharkley clay	83.6	74.7	69.3	82.0	73.7	66.9
Haldemand clay, subsoil	88.4	85.6	77.4	87.1	84.3	76.5

To ascertain the extent to which treatment with hydrogen perioxide effects the results obtained by the hydrometer method, soil samples were treated first with dilute and then with 30 per cent hydrogen peroxide and allowed to stand in a temperature of 65°C. for about 24 hours, with occasional stirring. The soils were then poured into the dispersing cup, stirred, and their mechanical analyses made by the hydrometer method. For comparison, check samples of the same soils were similarly analyzed by the hydrometer method, but without treatment. The comparative results obtained are shown in table 2.

The results in table 2 show that out of 28 soils studied, only in 2 did the treatment with hydrogen peroxide make a noticeable difference in their mechanical analyses. In all the other soils the treatment made hardly any difference; the small variations that are observed are within the limits of experimental error. The two soils in which the hydrogen peroxide affected the mechanical analysis are Beardon sandy loam and Fargo clay surface, both of

TABLE 2

Comparison of mechanical analyses of soils that did with those that did not receive peroxide treatment; both sets of soils being dispersed 10 minutes with the mechanical stirrer

	NOT TREATED			TREATED WITH HYDROGEN PEROXIDE		
	Colloids	Clay, 0.005–0.000 mm.	Fine clay, 0.002–0.000 mm.	Colloids	Clay, 0.005–0.000 mm.	Fine clay, 0.002–0.000 mm.
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Plainfield sand 0–6 inches	10.7	8.8	6.9	11.2	8.6	7.2
Plainfield sand 6–18 inches	9.6	7.0	5.5	9.8	7.8	6.3
Miles fine sandy loam	10.7	8.8	8.2	10.3	8.5	9.0
Kirven sandy loam	15.7	10.8	9.9	15.3	11.1	9.5
Beardon fine sandy loam, surface	19.6	16.8	13.7	21.8	19.7	18.6
Amarillo fine sandy loam	24.0	19.0	17.0	26.2	21.2	18.9
Loess soil 0–10 inches	25.6	14.5	12.9	27.0	18.6	16.0
Colby silt loam, surface	35.5	22.1	19.1	35.8	22.8	20.3
Colby silt loam, subsoil	35.0	25.5	19.0	36.2	25.1	21.1
Barnes loam	41.1	32.2	29.2	43.0	32.8	30.5
Cecil clay loam, subsoil, unlimed	42.2	37.7	36.5	43.5	38.1	35.7
Cecil clay loam, subsoil, limed	44.3	40.2	38.7	42.8	38.9	36.7
Ava silt loam	42.0	33.6	30.7	42.5	34.6	31.1
Grundy silt loam, subsoil	49.0	36.4	34.0	49.5	35.8	33.8
Lake Charles clay, surface	50.2	42.4	38.9	51.8	43.5	40.1
Lake Charles clay, subsoil	56.2	46.0	42.2	59.7	47.4	44.5
Aberdeen clay 4–10 inches	52.3	45.4	42.9	52.8	44.7	42.3
Welland clay, surface	59.8	49.5	42.5	61.2	52.0	43.8
Welland clay, subsoil	73.0	63.7	56.5	72.8	64.1	57.0
Fargo clay, surface	62.0	45.2	38.9	69.8	57.9	51.4
Fargo clay, subsoil	73.4	59.8	53.6	76.6	66.8	58.3
Osage clay	76.2	61.5	56.8	74.2	61.7	56.7
Parsons silt loam B	75.0	64.3	60.1	75.7	65.6	59.8
Parsons silt loam hardpan 24 inches	72.2	67.5	67.3	73.6	66.3	63.2
Kindersley clay 1–3 inches	77.6	75.9	72.2	77.4	76.3	71.7
Kindersley clay 3–8 inches	78.2	75.0	71.7	79.4	75.6	72.5
Sharkley clay	83.6	74.7	69.3	82.0	73.7	68.5
Haldemand clay, subsoil	88.4	85.6	77.4	89.1	82.7	76.2

which contain a very large amount of organic matter. From the data obtained with these two soils it would be logically expected that Lake Charles clay both surface and subsoil, Fargo clay subsoil, and Osage clay, all of which contain appreciable quantities of organic matter, would give similar results, but they did not. The probable explanation for this dissimilarity is that the former soils contain a larger amount of organic matter and in a more undecomposed state than the latter soils.

The indications are, therefore, that hydrogen peroxide treatment makes practically no difference in the dispersion of mineral soils containing no, or small amounts of, well-decomposed organic matter, but it makes considerable difference in soils that contain very large amounts of organic matter in an incompletely decomposed state.

STIRRING SOILS FOR DIFFERENT PERIODS WITH MECHANICAL STIRRER

The present procedure of the hydrometer method calls for stirring sands and extreme sandy loams for 5 minutes; loams, silt loams, clay loams, and clays, 10 minutes; and soils containing large amounts of undecomposed organic matter or soils that are recognized to be not easily dispersible, such as some of the lateritic soils and some claypans, 20 minutes or longer.

To measure the effect of long periods of stirring on the mechanical analyses of soils of various types, numerous samples were stirred for 10-minute and 60-minute periods and their mechanical analyses studied by the hydrometer method. The data are presented in table 3.

In general the data in table 3 show that stirring soils 60 minutes tends to increase their apparent content of finer material as compared with that at the 10-minute stirring. The increase, however, is most appreciable in the sands and sandy loams, and in some soils containing large amount of organic matter. In the clay loams and clays the increase is small, and in many cases can well be considered as being within the limits of experimental error.

With few possible exceptions, the increase in the finer material on long stirring is due almost entirely to the breaking up of the sand paricles. The speed of the stirrer (over 10,000 r.p.m.) together with the presence of wire baffles in the cup apparently tends to break up the sand particles. This view is supported by the greater relative increase in the fine material in the sandy soils, as compared with the clays, and also by the results in table 1, wherein it is shown that shaking sands for 16 hours has somewhat less dispersing effect on them than stirring them 10 minutes. It is on account of this possibility of breaking up the sand particles, that the hydrometer procedure (2) calls for stirring sandy soils only about 5 minutes.

The results in table 3 tend to indicate, therefore, that the period of stirring from about 5 to 10 minutes effects almost a complete dispersion of the great majority of soils. Undoubtedly there are some types of soils, such as the laterites, some claypans, and those containing large amounts of organic matter, especially of the undecomposed type, which require more than 10 minutes for complete dispersion. The studies presented herein, therefore, tend to show that the rapidity with which the hydrometer method measures the mechanical analyses of soils is not due to their incomplete dispersion.

The final procedure of the hydrometer method for determining total colloids in 15 minutes, clay (0.005–0.000 mm.) in 1 hour, and fine clay (0.002–0.000 mm.) in 2 hours, remains the same as previously published (2), except that soils containing very large amounts of organic matter especially of the undecomposed type should be treated with hydrogen peroxide. The further researches and comparisons of the hydrometer method have not caused any

TABLE 3

Comparison of mechanical analyses of soils dispersed by mechanical stirrer for 10-minute and 60-minute periods

	DISPERSED FOR 10 MINUTES			DISPERSED FOR 60 MINUTES		
	Colloids	Clay, 0.005–0.000 mm.	Fine clay, 0.002–0.000 mm.	Colloids	Clay, 0.005–0.000 mm.	Fine clay, 0.002–0.000 mm.
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Plainfield sand 0–6 inches	10.7	8.8	6.9	19.0	13.4	11.5
Plainfield sand 6–18 inches	9.6	7.0	5.5	14.1	11.9	9.8
Miles fine sandy loam	10.7	8.8	8.2	13.7	11.7	10.0
Kirven sandy loam	15.7	10.8	9.9	18.5	13.7	12.1
Beardon fine sandy loam, surface	19.6	16.8	13.7	24.4	21.9	20.3
Amarillo fine sandy loam	24.0	19.0	17.0	29.5	26.0	22.4
Loess soil 0–10 inches	25.6	14.5	12.9	30.1	19.6	16.4
Colby silt loam, surface	35.5	22.1	19.1	37.8	26.0	24.5
Colby silt loam, subsoil	35.0	25.5	19.0	37.5	28.2	24.1
Barnes loam	41.1	32.2	29.2	46.1	36.9	32.5
Cecil clay loam, subsoil, unlimed	42.2	37.7	36.5	44.8	39.2	36.8
Cecil clay loam, subsoil, limed	44.3	40.2	38.7	45.9	41.0	39.2
Ava silt loam	42.0	33.6	30.7	44.9	35.3	32.6
Grundy silt loam subsoil	49.0	36.4	34.0	51.3	40.8	38.0
Lake Charles clay, surface	50.2	42.4	38.9	51.3	44.1	41.6
Lake Charles clay, subsoil	56.2	46.0	42.1	58.6	47.3	43.2
Aberdeen clay 4–10 inches	52.3	45.4	42.9	53.7	48.8	45.5
Welland clay, surface	59.8	49.5	42.5	64.8	52.4	48.3
Welland clay, subsoil	73.0	63.7	56.5	73.9	65.8	59.8
Fargo clay, surface	62.0	45.2	38.9	70.3	57.4	51.2
Fargo clay, subsoil	73.4	59.8	53.6	77.4	68.0	59.9
Osage clay	76.2	61.5	56.8	77.3	63.5	57.3
Parsons silt loam B	75.0	64.3	60.1	75.5	67.5	62.7
Parsons silt loam hardpan 24 inches	72.2	67.5	67.3	75.5	68.7	66.8
Kindersley clay 1–3 inches	77.6	75.9	72.2	80.3	76.7	73.4
Kindersley clay 3–8 inches	78.2	75.0	71.7	81.2	76.7	73.2
Sharkley clay	83.6	74.7	69.3	85.5	77.4	71.7
Haldemand clay, subsoil	88.4	85.6	77.4	91.0	84.8	77.1

change in the original finding that the method is reasonably accurate and reliable for all practical purposes, and for the average person it is probably more reliable than any of the present methods.

SUMMARY

The hydrometer method of making mechanical analyses of soils was further investigated by comparing its mechanical stirrer with the standard shaker for dispersing soils. It was found that 10 minutes' stirring with the mechanical stirrer produced about the same degree of dispersion as 16 hours' shaking with the standard shaker.

Treating soils with hydrogen peroxide did not effect their mechanical analysis as measured by the hydrometer method except in soils that contain very large amounts of organic matter, especially of the undecomposed type.

On account of its efficiency, the mechanical stirrer tends to break down the sand particles on long stirring; hence it is advisable not to stir the sandy soils too long.

The extreme rapidity with which the hydrometer method is able to measure the mechanical analysis of soils is not due, therefore, to their incomplete dispersion.

Further research and comparison tend to support the original findings that, for all practical purposes, the hydrometer method is reasonably accurate and reliable.

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FURTHER STUDIES ON THE RELATIONSHIPS BETWEEN THE FINE MATERIAL OF SOILS AND THEIR PHYSICAL CHARACTERISTICS

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In previous communications (5) it was shown that certain soil characteristics, such as heat of wetting, moisture equivalent, and unfree water, bear a close relationship to that portion of the soil which is determined by the hydrometer method (2) at the end of 15 minutes' standing and which has been designated as "total soil colloids." On account of this close relationship it was revealed that these soil characteristics could be indirectly determined very quickly and simply by means of the hydrometer method.

In view of this promising study it was decided to investigate other soil characteristics and the relationship that they bear to the "total soil colloids" and to the conventional clay. One of the soil characteristics that was selected for detailed investigation is the consistency of soils. This characteristic or property of soils must be regarded of considerable importance, judging from the wide attention that it has received of late. It was thought that if the consistencies of soils bear a close relationship to the "total soil colloids" and to the clay as determined by the hydrometer method, then these consistencies could be indirectly determined very quickly and simply by the hydrometer method. It is apparent that this indirect determination would have decided advantage over the direct determination for the following reasons: (*a*) it is exceedingly rapid, (*b*) it is very simple, (*c*) it eliminates the personal element, and (*d*) it might be more reliable because the hydrometer method gives rather accurate results.

As is readily realized, any number of consistencies can be obtained in soils, especially the clays, by varying the moisture content. After preliminary examination and due consideration, three different consistencies were chosen for critical investigation; namely, the crumbling point, the flowing point, and the upper plastic limit of Atterberg (1). It is the object of this paper, therefore, to present the results obtained from the study of these consistencies. They will be considered separately. Further studies on moisture equivalent will also be incidently presented.

CRUMBLING POINT

The crumbling point represents that stage of moisture content at which the soil particle or crumbs have practically no cohesiveness—adhesiveness and crumble under slight pressure. This is the state at which soils under field conditions would work the easiest—they would plough easily and break to crumbs most readily and not stick to the implements. The moisture content at this crumbling point seems to be at or very close to the wilting coefficient of soils.

The procedure of determining this crumbling point of soils consists of placing about 20 gm. of air-dry soil that has passed through a 2-mm. sieve, into a porcelain dish, adding sufficient distilled water to wet it, and then allowing it to lose water at room temperature. When the soil loses enough water so that it approaches somewhat the crumbling point, it is transferred to a paper on the table and rubbed with the palm of the hand in a circulatory motion. If the soil is near its crumbling point, it is easily reduced to crumbs or compound particles by this rubbing process. Then a small amount of soil is taken and rolled between the fingers and then this rolled piece of soil is taken end by end and pressed between the fingers. If the soil has reached the proper moisture content the rolled piece will readily crumble into crumbs without showing any stickiness or cohesiveness. This is the approximately true crumbling point. If the soil has not reached the proper moisture content the crumbs will tend to stick and do not fall apart easily. In this case, the soil is allowed to lose more moisture as it is being rubbed and mixed on the paper, and is tested often to see if the proper moisture content is reached.

The procedure is of course emperical and would seem to involve considerable personal element, but the transitional point is sufficiently definite to yield closely reproducible results. With little experience one can get good agreements. Extensive tests showed that for the majority of soils results could be reproduced or checked, even at different dates, within 2 per cent.

In table 1 are reported the crumbling points of a fairly large number of representative types of soils coming from many regions.[1] In the same table are also reported the mechanical analysis of "total colloids," clay, and fine clay, as obtained by the hydrometer method (4) and the relationships that these soil fractions bear to the crumbling point.

The last three columns of table 1 readily show that there is a close relationship between the total colloid, clay, fine clay, and the crumbling point. The coefficient of correlation is 0.966 ± 0.013 for the "total colloid," 0.943 ± 0.021 for the clay, and 0.922 ± 0.029 for the fine clay. Reducing these coefficients of correlation to percentages by the process of squaring the R-values and then multiplying by 100, shows how much of the variability in crumbling point is caused by each soil fraction. These calculations show that the variability in crumbling point is influenced 93.8 per cent by the total colloids, 88.9

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[1] The writer is grateful for soil samples received to: C. F. Marbut—U. S. Bureau of Soils, E. A. Norton—University of Illinois, A. H. Joel—University of Saskatchewan, A. S. Cline—North Carolina Agricultural College, E. B. Reynolds—Texas Agricultural College, and G. N. Ruhnke—Ontario Agricultural College.

per cent by the clay, and 85.0 per cent by the fine clay. Evidently the crumbling point of soils is almost entirely controlled by their fine material. The influence exerted by other factors such as the nature of the fine soil material is comparatively small.

TABLE 1

Relationship between crumbling point of soils, and their content of total colloids, clay, and fine clay

	TOTAL COLLOIDS	CLAY, 0.005–0.000 mm.	FINE CLAY, 0.002–0.000 mm.	CRUMBLING POINT	RATIO CRUMBLING POINT / TOTAL COLLOIDS	RATIO CRUMBLING POINT / CLAY	RATIO CRUMBLING POINT / FINE CLAY
	per cent	*per cent*	*per cent*	*per cent*			
Amarillo fine sandy loam 7–18 inches	24.0	19.0	17.0	6.5	0.2708	0.3421	0.3823
Loess soil 0–10 inches	25.6	14.5	13.0	7.4	0.2891	0.5103	0.5692
Stewart sandy loam B	28.6	20.5	14.0	7.4	0.2587	0.3609	0.5285
Colby silt loam, surface	35.0	22.1	19.1	10.0	0.2857	0.4524	0.5235
Colby silt loam, subsoil	35.0	25.5	19.0	9.2	0.2628	0.3608	0.4842
Barnes loam 20–40 inches	41.1	32.2	29.2	11.5	0.2798	0.3571	0.3938
Ava silt loam, subsoil	42.0	33.6	30.7	10.9	0.2595	0.3244	0.3550
Cecil clay loam, subsoil, unlimed	42.2	37.7	36.5	11.5	0.2725	0.3052	0.3151
Cecil clay loam, subsoil, limed	44.3	40.2	38.7	11.8	0.2655	0.2935	0.3050
Davison loam 0–9 inches	42.4	35.9	31.0	11.4	0.2688	0.3175	0.3677
Marshall silt loam 4–18 inches	45.3	35.6	31.4	13.2	0.2913	0.3707	0.4204
Grundy silt loam, subsoil	49.0	36.5	34.0	14.0	0.2857	0.3861	0.4117
Lake Charles clay, surface	50.2	42.4	38.9	13.4	0.2669	0.3160	0.3444
Lake Charles clay, subsoil	56.2	46.0	42.1	13.1	0.2331	0.2847	0.3111
Soil from Fallon, Nevada	49.9	40.6	36.3	13.9	0.2785	0.3424	0.3824
Aberdeen clay 4–10 inches	52.3	45.4	42.9	12.2	0.2332	0.2687	0.2843
Welland clay, surface	59.8	49.5	42.5	17.7	0.2959	0.3575	0.4164
Welland clay, subsoil	73.0	63.7	56.5	20.8	0.2849	0.3265	0.3681
Fargo clay, surface	69.6	57.9	51.4	21.0	0.3017	0.3626	0.4085
Fargo clay, subsoil	76.6	66.8	58.2	20.6	0.2689	0.3084	0.3539
Kinderly clay 1–3 inches	77.6	75.9	72.2	19.3	0.2487	0.2543	0.2673
Kinderly clay 3–8 inches	78.2	75.0	71.7	19.9	0.2544	0.2653	0.2775
Parsons silt loam B	75.0	64.3	60.1	17.9	0.2386	0.2783	0.2978
Osage clay	76.2	61.5	56.8	18.2	0.2388	0.2959	0.3204
Sharkley clay	83.6	74.7	69.3	20.5	0.2452	0.2744	0.2958
Miami silty clay loam 14–18 inches	80.0	68.3	63.0	19.0	0.2437	0.2855	0.3095
Haldemand clay, subsoil	88.4	85.6	77.4	20.6	0.2335	0.2412	0.2667
Average ratio					0.2617	0.3092	0.3048
Coefficient of correlation					0.966	0.943	0.922
Coefficient of correlation reduced to percentage basis					93.3	88.9	85.0
Probable error					0.013	0.021	0.029

In view of these close relationships the crumbling point of any soil can be determined indirectly and quickly by means of the hydrometer method. This can be accomplished according to the following formulas:

$$\frac{\text{Total colloids}}{\text{Average}\,\dfrac{\text{Crumbling point}}{\text{Total colloids}}\,\text{Ratio}} = \text{Crumbling point}$$

$$\frac{\text{Clay}}{\text{Average}\,\dfrac{\text{Crumbling point}}{\text{Clay}}\,\text{Ratio}} = \text{Crumbling point}$$

$$\frac{\text{Fine clay}}{\text{Average}\,\dfrac{\text{Crumbling point}}{\text{Fine clay}}\,\text{Ratio}} = \text{Crumbling point}$$

Since the "total colloids" formula involves only 15 minutes and it seems to give results just as good as, or better than, the clay and fine clay formulas which require 1 and 2 hours respectively, then the "total colloid" formula can be preferably used to determine the crumbling point of soils.

FLOWING POINT

The flowing point was chosen to represent one of the extremes toward the wet side of the soil as the crumbling point was selected to represent one of the extremes on the dry side.

In order to reduce the results to a comparable and uniform basis the soils were subjected to dispersion.

The final procedure adopted for measuring the flowing point of soils consists of placing about 50 gm. of soil into a 100-cc. beaker, adding sufficient distilled water to saturate it, and then mixing it and dispersing it by means of the motor shown in plate 1. The dispersion is continued with the addition of small amounts of water until the mixture commences to flow, when the beaker is turned to an angle and the mixture does not thicken on further dispersion.

The soil is dispersed for at least 15 minutes. The more the soil is dispersed the more water it requires to come to the flowing point. When it is about completely dispersed, additional stirring does not change its consistency or flowing point.

The process of dispersion tends to increase greatly the amount of water that many clay soils will take up to reach the flowing point. In some instances it is almost 100 per cent increase, whereas in other soils the increase is small.

In table 2 are presented the results on flowing point of the same soils as used in the crumbling point studies. In the same table is shown the relationship that exists between the flowing point of the various soils and their "total colloid," clay, and fine clay.

The results in table 2 show that there is a good correlation between the flowing point and the "total colloid," clay, and fine clay of the various soils.

The coefficient of correlation for all of the soils is .921 ± .029 for the "total colloid," .906 ± .034 for the clay, and .915 ± .031 for the fine clay. When these figures are expressed on a percentage basis it is seen that the variability in the flowing point of soils is influenced about 84.8 per cent by their "total colloids," 82.1 per cent by their clay content, and about 83.7 per cent by their fine clay content.

TABLE 2

Relationships between the finer material of soils and their flowing points*

	FLOWING POINT	RATIO $\frac{\text{F. P.}}{\text{TOTAL COLLOIDS}}$	RATIO $\frac{\text{F. P.}}{\text{CLAY}}$	RATIO $\frac{\text{F. P.}}{\text{FINE CLAY}}$
	per cent			
Amarillo fine sandy loam 7–18 inches	57.3	2.3875	3.0157	3.3705
Loess soil 0–10 inches	41.9	1.6367	2.8896	3.2230
Stewart sandy loam B	38.4	1.3426	1.8731	2.7428
Colby silt loam, surface	50.1	1.4314	2.2669	2.6231
Colby silt loam, subsoil	55.5	1.5857	2.1764	2.9210
Barnes loam 20–40 inches	72.0	1.7516	2.2360	2.4657
Ava silt loam, subsoil	81.0	1.9285	2.4107	2.6384
Cecil clay loam, subsoil, unlimed	65.3	1.5473	1.7320	1.7890
Cecil clay loam, subsoil, limed	65.3	1.4740	1.6243	1.6873
Davison loam 0–9 inches	77.5	1.8278	2.1587	2 5000
Marshall silty loam 14–18 inches	93.4	2.0618	2.6235	2.9745
Grundy silt loam, subsoil	90.0	1.8367	2.4724	2.6239
Lake Charles clay, surface	89.3	1.7788	2.1061	2.2956
Lake Charles clay, subsoil	101.8	1.8113	2.2130	2.4181
Soil from Fallon, Nevada	88.4	1.7715	2.1773	2.4352
Aberdeen clay 4–10 inches	93.1	1.7801	2.0506	2.1701
Welland clay, surface	81.5	1.3628	1.6464	1.9176
Welland clay, subsoil	120.0	1.6438	1.8838	2.1238
Fargo clay, surface	81.0	1.164	1.3989	1.4336
Fargo clay, subsoil	106.8	1.4550	1.7859	1.9925
Kinderley clay 1–3 inches	130.5	1.6817	1.7193	1.8074
Kinderley clay 3–8 inches	118.0	1.5089	1.5733	1.6457
Parsons silt loam B	126.2	1.6826	1.9626	2.0998
Osage clay	107.0	1.4041	1.7398	1.8838
Sharkley clay	128.8	1.5406	1.7242	1.8585
Miami silty clay loam 14–18 inches	104.0	1.3870	1.5226	1.6507
Haldemand clay subsoil	134.2	1.5180	1.5677	1.7338
Average ratio		1.6071	1.8977	2.0788
Coefficient of correlation		0.921	0.906	0.915
Coefficient of correlation reduced to percentage basis		84.80	82.10	83.70
Probable error		0.029	0.034	0.031

* Percentages of total colloids of clay 0.005–0.000 mm., and of fine clay 0.002–0.000 mm. are given in table 1.

Although these figures indicate a very good correlation when all the soils are taken as a whole, the ratios shown in the last three columns of the table reveal that in a few cases there is considerable individual variation in the relationship. This occasional individual variation suggests that the property of flowing is not controlled in all soils as completely by the "colloidal" or clay content as are some of the other properties, such as the crumbling point and moisture equivalent.

Probably the chemical composition of a soil would tend to influence the flowing point because some soils of certain composition or with certain salts present would lend themselves to emulsification and others would not. For instance, Parsons silt loam of the C horizon is derived from fuller's earth, and any soil that is derived from fuller's earth or from bentonite tends to emulsify and gelatinize and thereby take up a large amount of water to reach the flowing point. On the other hand, soils such as Cecil loam show practically no emulsification and consequently do not take up as much water to reach the flowing point.

On account of this individual variation of certain soils, it is probably not safe to apply a formula to determine the flowing point for all soils. According to the results in table 2, the flowing point of the vast majority of soils can be determined comparatively accurately by the indirect method shown in the following but for some soils the indirect determination may not be so accurate. The formulas for the indirect determination are as follows:

$$\frac{\text{Total colloids}}{\text{Average}\,\dfrac{\text{Flowing point}}{\text{Total colloids}}\,\text{Ratio}} = \text{Flowing point}$$

$$\frac{\text{Clay}}{\text{Average}\,\dfrac{\text{Flowing point}}{\text{Clay}}\,\text{Ratio}} = \text{Flowing point}$$

$$\frac{\text{Fine clay}}{\text{Average}\,\dfrac{\text{Flowing point}}{\text{Fine clay}}\,\text{Ratio}} = \text{Flowing point}$$

The flowing point appears to possess considerable significance in that it may prove to be very useful in distinguishing and selecting clays for various purposes in the general ceramic industry. In a subsequent paper it will be shown that it can be used to distinguish the various kinds of bentonites.

UPPER PLASTIC LIMIT

The conventional procedure (1) was used to measure the upper plastic limit of the same soils that have been used for the crumbling point and flowing point. The general results are detailed in table 3.

The results in table 3 also show that there is a strong tendency for the "total colloids," clay, and fine clay to control the plasticity of soils. The coefficient of correlation for all the soils is .916 ± .031 for the "total colloids," .914 ± .032 for the clay, and .920 ± .030 for the fine clay. In other words, the variability of the upper plastic limit of soils seems to be influenced to the extent of about 83.9 per cent by the total colloids, 83.5 per cent by the clay, and 84.6 per cent by the fine clay.

TABLE 3

Relationships between the finer materials of soils and their upper plastic limits*

	UPPER PLASTIC LIMIT	RATIO U. P. L. / TOTAL COLLOIDS	RATIO U. P. L. / CLAY	RATIO U. P. L. / FINE CLAY
	per cent			
Amarillo fine sandy loam 7–18 inches	18.6	0.7750	0.9789	1.0947
Loess soil 0–10 inches	22.0	0.8590	1.5170	1.7100
Stewart sandy loam B	17.1	0.5980	0.8340	1.222
Colby silt loam, surface	27.7	0.7914	1.2533	1.4502
Colby silt loam, subsoil	22.5	0.6428	0.8823	1.1842
Barnes loam 20–40 inches	27.3	0.6645	0.8480	0.9350
Ava silt loam, subsoil	30.7	0.7309	0.9136	1.0000
Cecil clay loam, subsoil, unlimed	26.5	0.6279	0.7029	0.7260
Cecil clay loam, subsoil, limed	25.4	0.5733	0.6318	0.6563
Davison loam 0–9 inches	29.7	0.7004	0.8272	0.9580
Marshall silt loam 14–18 inches	40.7	0.8984	1.1432	1.2961
Grundy silt loam, subsoil	27.7	0.5653	0.7909	0.8076
Lake Charles clay, surface	39.6	0.7888	0.9339	1.0179
Lake Charles clay, subsoil	45.9	0.8167	0.9978	1.0902
Soil from Fallon, Nevada	29.5	0.5911	0.7266	0.8127
Aberdeen clay 4–10 inches	43.9	0.8393	0.9669	1.0233
Welland clay, surface	40.3	0.6740	0.8141	0.9482
Welland clay, subsoil	43.2	0.5920	0.6782	0.7645
Fargo clay, surface	48.9	0.7025	0.8455	0.8655
Fargo clay, subsoil	49.3	0.6716	0.8244	0.9198
Kindersley clay 1–3 inches	63.1	0.8131	0.8315	0.8739
Kindersley clay 3–8 inches	64.9	0.8299	0.8653	0.9052
Parsons silt loam B	55.0	0.7333	0.8553	0.9151
Osage clay	47.4	0.6221	0.7707	0.8345
Sharkley clay	61.9	0.7404	0.8286	0.8932
Miami silty clay loam 14–18 inches	46.7	0.6230	0.6837	0.7412
Haldemand clay subsoil	54.8	0.6199	0.6401	0.7080
Average ratio		0.7035	0.8311	0.9104
Coefficient of correlation		0.916	0.914	0.920
Coefficient of correlation reduced to percentage basis		83.90	83.50	84.60
Probable error		0.031	0.032	0.030

* Percentages of total colloids, of clay 0.005–0.000 mm., and of fine clay 0.002–0.000 mm. are given in table 1.

Although these are good correlations for all the soils as a whole, an examination of the last three columns shows that some individual soils exhibit considerable variation from the majority.

The formulas for measuring the upper plastic limit of soils indirectly by means of the hydrometer method are as follows:

$$\frac{\text{Total colloids}}{\text{Average }\dfrac{\text{Upper plastic limit}}{\text{Total colloids}}\text{ Ratio}} = \text{Upper plastic limit}$$

$$\frac{\text{Clay}}{\text{Average }\dfrac{\text{Upper plastic limit}}{\text{Clay}}\text{ Average ratio}} = \text{Upper plastic limit}$$

$$\frac{\text{Fine clay}}{\text{Average }\dfrac{\text{Upper plastic limit}}{\text{Fine clay}}\text{ Average ratio}} = \text{Upper plastic limit}$$

MOISTURE EQUIVALENT

The method described in a previous communication (3) was used to measure the moisture equivalent of the same soils that were employed in the preceding studies. In table 4, are presented the general results obtained.

The data in table 4 show good correlations between the moisture equivalent and the "total colloids," clay and fine clay. The correlation for the total colloids is .909 ± .033, for the clay is .895 ± .038, and for the fine clay is .897 ± .038. Reducing these figures to the percentage basis they indicate that the variability of the moisture equivalent is influenced to the extent of about 82.7 per cent by the total colloids, 80.1 per cent by the clay, and 80.5 per cent by the fine clay.

The formulas for determining the moisture equivalent of soils indirectly by means of the hydrometer method are as follows:

$$\text{Colloids} \times \frac{\text{Moisture equivalent}}{\text{Colloids}}\text{ Average ratio} = \text{Moisture equivalent}$$

$$\text{Clay} \times \frac{\text{Moisture equivalent}}{\text{Clay}}\text{ Average ratio} = \text{Moisture equivalent}$$

$$\text{Fine clay} \times \frac{\text{Moisture equivalent}}{\text{Fine clay}}\text{ Average ratio} = \text{Moisture equivalent}$$

In view of the variations that some individual soils show in some of the preceding correlations, it was desired to ascertain whether these variations were due to differences in chemical compositions of the respective soils. Accordingly, the very fine material of several of the soils which showed variations, was analyzed[2] for silica, aluminum, and iron. The fine material analyzed was

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[2] The chemical analysis was made by O. B. Winter of this station.

TABLE 4

Relationship between the finer material of soils and their moisture equivalents*

	MOISTURE EQUIVALENT	RATIO $\frac{\text{M. E.}}{\text{TOTAL COLLOIDS}}$	RATIO $\frac{\text{M. E.}}{\text{CLAY}}$	RATIO $\frac{\text{M. E.}}{\text{FINE CLAY}}$
Amarillo fine sandy loam 7–18 inches	19.4	0.8083	1.021	1.1411
Loess soil 0–10 inches	18.4	0.7187	1.268	1.4150
Stewart sandy loam B	16.5	0.5770	0.8050	1.1780
Colby silt loam, surface	24.9	0.7114	1.1266	1.303
Colby silt loam, subsoil	26.2	0.7485	1.027	1.379
Barnes loam 20–40 inches	21.5	0.5231	0.6677	0.7363
Ava silt loam, subsoil	24.0	0.5713	0.7140	0.7765
Cecil clay loam, subsoil, unlimed	24.2	0.5734	0.6419	0.6630
Cecil clay loam, subsoil, limed	24.8	0.5598	0.6169	0.6408
Davison loam 0–9 inches	28.0	0.6603	0.7799	0.9032
Marshall silty loam 14–18 inches	33.0	0.7284	0.9269	1.051
Grundy silt loam, subsoil	35.0	0.7142	0.9615	1.029
Lake Charles clay, surface	39.5	0.7868	0.9316	1.052
Lake Charles clay, subsoil	42.3	0.7526	0.9195	1.005
Soil from Fallon, Nevada	38.5	0.7712	0.9482	1.061
Aberdeen clay 4–10 inches	38.8	0.7418	0.8546	0.9044
Welland clay, surface	36.7	0.6137	0.7414	0.8635
Welland clay, subsoil	39.5	0.5412	0.6200	0.6990
Fargo clay, surface	53.5	0.7686	0.9240	1.0408
Fargo clay, subsoil	42.0	0.5483	0.6287	0.7216
Kindersley clay 1–3 inches	51.4	0.6623	0.6772	0.7119
Kindersley clay 3–8 inches	61.8	0.7902	0.8240	0.8619
Parsons silt loam B	48.5	0.6466	0.7542	0.8069
Osage clay	50.0	0.6561	0.8130	0.8802
Sharkley clay	53.0	0.6339	0.7095	0.7647
Miami silty clay loam 14–18 inches	43.0	0.5374	0.6295	0.6830
Haldemand clay subsoil	48.5	0.5486	0.5665	0.6266
Average ratio		0.6550	0.7734	0.8525
Coefficient of correlation		0.909	0.895	0.897
Coefficient of correlation reduced to percentage basis		0.8270	0.8010	0.8050
Probable error		0.033	0.038	0.038

* Percentages of total colloids, of clay 0.005–0.000 mm., and of fine clay 0.002–0.000 mm. are given in table 1.

TABLE 5

Comparison of physical properties of soils with their chemical compositions

	SiO_2	$Fe_2O_3+Al_2O_3$	Fe_2O_3	Al_2O_3	CRUMBLING POINT	FLOWING POINT	UPPER PLASTIC LIMIT	MOISTURE EQUIVALENT
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Cecil clay loam 7–18 inches	39.2	49.2	12.4	36.8	11.5	65.3	26.5	24.2
Welland clay subsoil	47.84	40.06	13.01	27.05	20.8	120.0	43.2	39.5
Aberdeen clay 4–10 inches	50.65	23.70	7.44	16.26	12.2	93.1	43.9	38.8
Barnes loam	49.68	28.20	6.63	21.57	11.5	72.0	27.3	21.5
Haldemand clay, subsoil	50.50	28.10	8.81	19.29	20.6	134.2	54.8	48.5
Osage clay	52.01	27.50	8.16	19.34	18.2	128.0	47.4	50.0
Kindersley clay 1–3 inches	53.01	24.75	7.71	17.04	19.3	130.5	63.1	51.4
Fargo clay, surface	52.54	20.30	8.08	12.22	13.4	106.8	48.9	53.5
Amarillo fine sandy loam	50.91	30.39	8.31	22.08	6.5	57.3	18.6	19.4

obtained from soil suspension which had stood for 30 hours. In table 5 are shown the chemical compositions of the fine material of a number of soils together with their crumbling point, flowing point, upper plastic limit, and moisture equivalent for comparison.

The data in table 5 reveal that there is no definite relationship between the physical properties of the soils studied and the content of aluminum, silica, and iron of their fine material. The variations in the correlations of individual soils, therefore, cannot be explained on the basis of differences of these constituents in the fine material of these soils. These findings, however, do not contradict the results of other investigators who have found that there is a relationship between certain physical properties of soils as a whole and their chemical composition.

Referring once again to tables 1, 2, 3, and 4, one will see that the "total colloids"—the soil material determined at 15 minutes standing by the hydrometer method—shows a closer correlation with the various soil physical properties studied than does either the conventional clay or the fine clay. These results tend to confirm the views previously expressed that the "total colloids" are more representative of the nature and texture of the soil material that controls the physical properties of soils, than is the clay, fine clay, or true colloids.

Finally it should be emphasized that although the soils employed give a fairly representative list of different types, undoubtedly there may be some types or individual soils which will not show as good correlations between the fine material and physical characteristics as are revealed in the foregoing. The sands, mucks, and peats, for instance, do not show such good correlations, nor would it be expected.

SUMMARY

A study was made to ascertain whether there are close relationships between the "total colloids," clay, and fine clay of soils as determined by the hydrometer method, and certain consistencies of soils such as the crumbling point, flowing point, and upper plastic limit. The idea underlying this investigation was that, if there do exist such close relationships, then the consistencies and other soil physical properties can be indirectly determined very quickly and simply and probably more accurately by means of the hydrometer method.

From the results obtained coefficients of correlation were worked out, and these in turn were reduced to the percentage basis.

The coefficients of correlation on the soils worked show that the variability of the crumbling point is controlled 93.3 per cent by the "total colloids," 88.93 per cent by the clay, and 85.0 per cent by the fine clay.

The variability of the flowing point is controlled 84.8 per cent by the "total colloids," 82.1 per cent by the clay, and 83.7 per cent by the fine clay.

The variability of the upper plastic limit is controlled 83.9 per cent by the "total colloids," 83.5 per cent by the clay, and 84.6 per cent by the fine clay.

The variability of the moisture equivalent is influenced 82.7 per cent by the "total colloids," 80.1 per cent by the clay, and 80.5 per cent by the fine clay.

No definite relationship could be established between the chemical composition of the fine material of soils studied and their physical properties studied.

Undoubtedly there may be some types or individual soils which will not show as good correlation between the fine material and physical characteristics as revealed in soils studied here.

From the correlations obtained it would seem that the hydrometer method can be employed to obtain indirectly and quickly certain physical characteristics of soils.

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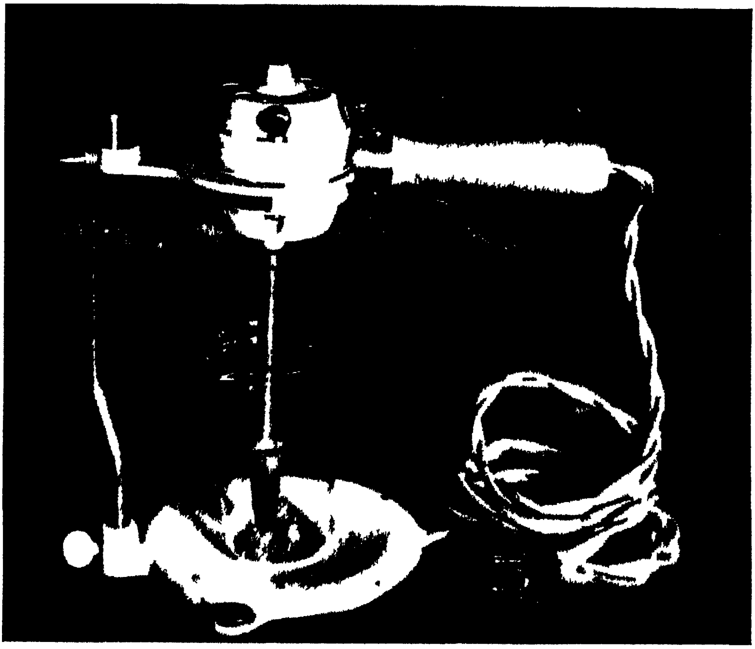
PLATE 1

Machine Used to Disperse Soils in Determining the Flowing Point of Soils



THE LAWS OF SOIL COLLOIDAL BEHAVIOR: VII. PROTEINS AND PROTEINATED COMPLEXES[1]

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Among the inorganic soil colloids we have distinguished between two types of ampholytoids. In the first place we have the amphoteric sesquioxides, which may be classified as primary ampholytoids. Then there is a practically infinite number of amphoteric complexes, derived from the sesquioxides, which constitute insoluble salts with weak acids such as silicic, phosphoric, and humic acids. These might be looked upon as secondary ampholytoids. The law governing their formation and their behavior has been the subject of the last four sections of this series (9, 10, 11, 12).

Among the organic colloids of the soil we meet with a similar grouping of materials. Here the primary ampholytoids are represented by the "pure" proteins whereas the secondary organic ampholytoids constitute an endless number of protein compounds or, what we have chosen to call, proteinated complexes. It is these complexes which form the subject matter of this paper.

Although the isoelectric pH of the hydroxides of Al and Fe [about 8.1 and 7.1 respectively when precipitated from the chlorides by NaOH (9)] is so high that these ampholytoids play their most important rôle in the soil as basoids, the isoelectric pH of many of the proteins is such as to bring out their dual character within the usual range of soil reaction. Michaelis (13) gives 4.7 as the isoelectric pH of the three proteins casein, serum albumin, and gelatin. Edestin, as we shall see, has a higher isoelectric pH, or about 5.2. Edestin is therefore a stronger base and a weaker acid than either of the former proteins. Such differences are probably due to the relative proportions of the various amino acids and their form of linkage in the protein molecules. A protein containing a high proportion of the diamino-mono carboxylic acids arginine and lysine might be expected to be more basic and less acid and to have a higher isoelectric pH than proteins having a low content of these constituents. On the other hand a protein containing a high proportion of the mono-amino di-carboxylic glutamic and aspartic acids might be expected to be more acid and to have a lower isoelectric pH. Thus the protamine salmin, which is reported to contain as high as 88 per cent arginine, possesses an alkaline reaction and would undoubtedly show a very high isoelectric pH, whereas gliadin, which contains as high as 42 per cent glutamic acid, is one of the most acid proteins and is probably isoelectric at a comparatively low pH.

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[1] Paper of the Journal Series, New Jersey Agricultural Experiment Station, department of soil chemistry and bacteriology.

In the present work it would have been highly desirable to study proteins representing a wide variation in their acid and basic properties but unfortunately none but the more common ones; namely, egg albumen, casein, edestin, and gelatin were available. Of these four proteins edestin, however, differs sufficiently in its acid and basic properties and in its isoelectric pH to bring out fundamental differences in amphoteric behavior.

THE BUFFER CAPACITY AND THE ELECTROKINETICS OF THE ("FREE") PROTEINS

The various proteins were first studied as follows: One gram protein was dissolved in a solution containing 1 m. mol NaOH and diluted to 1 liter. Twenty cubic centimeter portions of the protein solutions were mixed with 30 cc. water containing various amounts of HCl. The pH was determined colorimetrically where there was no interference by color reactions. Where this was the case the determinations were made by the quinhydrone electrode. The cataphoresis measurements were made by the ultra-microscopic method as previously described (6).

Gelatin, as is well known, does not coagulate even at the isoelectric point. No gelatin particles were therefore visible in the ultramicroscope. This is also true of the other proteins except at or near the isoelectric point, for these all form an isoelectric floc. Where no particles were visible the technique previously employed (5) was resorted to. This consists in adding a very small amount of an inert powder such as pumice. It appears as if the pumice particles coat themselves with the protein and behave electrokinetically as if they consisted of protein. No difference in cataphoresis was observed when about 10 times the necessary amount of pumice was added. Nor is there a break in the curves of casein, edestin, and albumin near the isoelectric point where no pumice was added. The method is therefore justified both by the results and by the fact that there is at present no other way of directly observing the cataphoretic movement of ultramicroscopically invisible particles. [The method of The Svedberg (14) who photographed the movement of the protein solution by the use of ultraviolet light would be too elaborate where a great number of measurements are to be made.]

The proteins, which were of the kind supplied by chemical firms, had the following composition:

NAME OF PROTEIN	EGG ALBUMIN (EIMER AND AMEND)	CASEIN (HARRIS)	EDESTIN C.P. (EIMER AND AMEND)	BACTO GELATIN ("DIFCO" STANDARD)
Water, per cent, at 105°C.	10.72	8.86	7.66	12.45
Ash, per cent	3.25	3.09	1.06	2.43
N, per cent, 105° basis	14.33	15.67	17.67	18.06

The results of the experiments are shown in table 74 and in figure 11.

The electrokinetic behavior of the four proteins shows significant differences. All, except gelatin, coagulate over a narrow zone at the isoelectric pH. The albumin was isoelectric at pH 4.95, and the casein was, by interpolation, isoelectric at a pH slightly above 4.8. Edestin and gelatin were both found isoelectric at about 5.2.

TABLE 74

Cataphoresis of albumin, casein, edestin, and gelatin (20 cc. of a solution containing 1 gm. protein + 1 millimol NaOH in 1,000 cc.)

0.01 N HCl*	ALBUMIN			CASEIN			EDESTIN			GELATIN		
	Floc	μ/sec. 1 volt/cm.	pH	Floc	μ/sec. 1 volt/cm.	pH	Floc	μ/sec. 1 volt/cm.	pH	Floc	μ/sec. 1 volt/cm.	pH
cc.												
0.4	0	−2.75	9.6	+	−2.75	8.4	0	−3.03	8.9	0	−1.38	9.1
0.8	0	−2.63	9.2	+	−2.36	7.1	0	−3.03	8.4	0	−1.01	8.7
1.6	0	−2.52	6.8	+	−2.16	5.9	+	−2.63	6.2	0	−0.67	5.95
2.0	....	......	....	....	......	....	+++	−1.08	5.6	0	−0.43	5.4
2.2	....	......	....	....	......	....	++++	−0.76	5.3	0	±0.0	5.2
2.4	....	......	....	++	−1.89	5.2	+++	+0.74	5.1	0	+0.42	5.0
2.6	++++	−0.38	5.1	+++	−1.68	5.0	++	+1.51	5.0	0	+0.61	4.7
2.7	++++	±0.0	4.95	....	......	....	....	......	....	...	......	....
2.8	++++	+0.20	4.85	++++	−0.61	4.9	++	+1.89	4.95	0	+0.78	4.5
3.0	+++	+0.52	4.75	++++	+0.15	4.8	....	......	....	...	......	....
3.2	0	+0.95	4.60	+++	+0.76	4.7	+	+2.33	4.9	0	+1.21	4.15
3.6	0	+1.42	4.45	++	+1.12	4.45	....	......	....	...	......	....
6.4	0	+2.02	4.2	++	+1.32	4.2	+	+2.52	4.5	0	+1.32	3.4
12.8	0	+2.20	....	+	+2.08	3.8	0	......	4.3	0	+1.38	2.8
25.6	0	+2.64	....	+	+2.52	3.5	0	+2.75	3.8	0	+1.51	2.4

0 = clear; + = opalescent; ++ = dense opal; +++ = milky; ++++ = flocculation.

* Plus enough water to make 50 cc.

The casein was studied in greater detail. Thus when 2.9 cc. 0.01 N HCl was added to the casein solution, in duplicate tubes, the pH was 4.8 and the protein slightly negative. When H_2SO_4 and H_3PO_4 were added to the Na-caseinate instead of HCl the results were as follows:

0.01 N H_2SO_4	FLOC	μ/SEC.	pH
cc.			
2.9	++++	−0.24	4.8
3.0	++++	+0.24	4.7
0.01 M H_3PO_4			
cc.			
2.9	++++	−0.24	4.8
3.0	++++	+0.16	4.7

This all indicates an isoelectric point between 4.7 and 4.8 and that the casein "sulfate" and "phosphate" are as soluble as the "chloride." The common

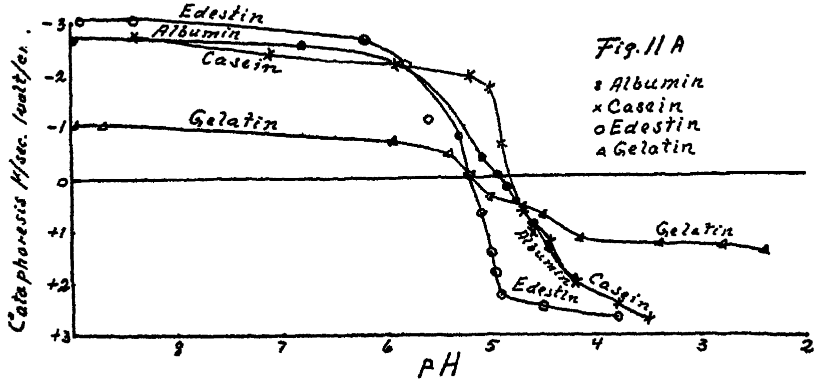


FIG. 11A. CATAPHORESIS OF ALBUMIN, CASEIN, EDESTIN, AND GELATIN AT DIFFERENT pH VALUES, ADJUSTED BY NaOH AND HCl

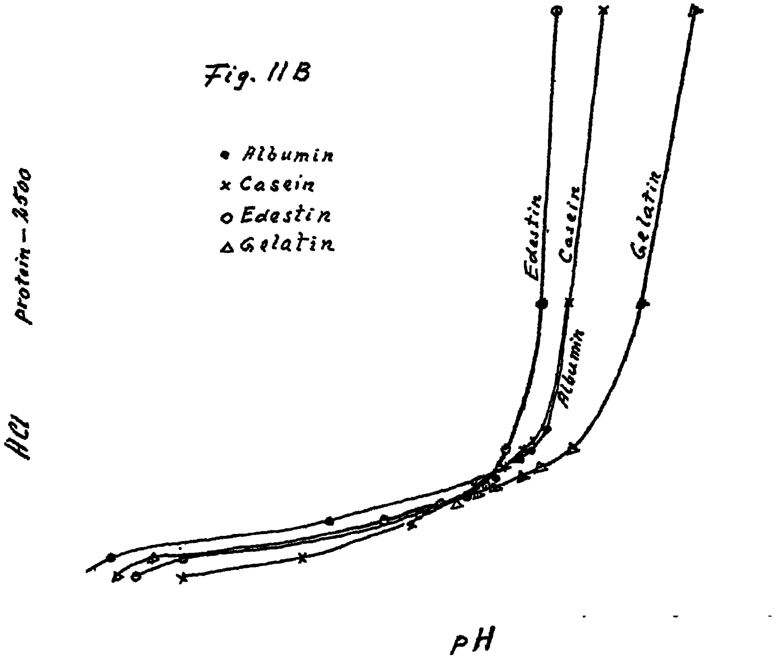


FIG. 11B. THE BUFFER CAPACITY OF EDESTIN, ALBUMIN, CASEIN, AND GELATIN IN HCl SOLUTION

The original solutions contained 1 gm. protein + 1 millimol NaOH in 2,500 cc.

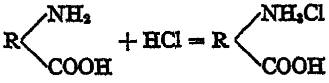
mineral anions do not, therefore, affect the isoelectric point of the proteins. (The isoelectric point of the sesquioxides is, as we have seen, greatly displaced to the acid side by the sulfate and especially by the phosphate ions. This we ascribed to a decrease in the anionic dissociation of the sulfated and phosphated complexes.)

Apart from differences in the isoelectric points it was found that gelatin shows a distinctly different behavior from the other proteins. Thus the three proteins albumen, casein, and edestin become strongly negative and positive on the respective sides of the isoelectric point while gelatin remains electrokinetically very sluggish over the entire range. This is most clearly brought out by the flattened form of the gelatin curve in figure 11A. This would lead us to suspect that gelatin is less reactive both as an acid and as a base.

The curves in figure 11B express the buffer capacity of the four proteins. Albumen and casein show about the same capacity to neutralize acids whereas edestin is a decidedly stronger base which is in harmony with the higher isoelectric point of this protein. Gelatin, which was found isoelectric at a pH (5.2) as high as that of edestin shows the weakest buffer action for acids. This again, points to a less active protein. In our study of the proteinated complexes we shall find a consistent indication of this relative inactivity of gelatin. This property of gelatin might be related to its origin, namely, the connective tissues (collagen). Figure 11B does not bring out significant differences in the buffer action toward bases. To show this the pH must be brought to higher values, for although the proteins here studied are stronger as acids than as bases they are yet too weak as acids to neutralize much base except in alkaline solutions. The curves run accordingly more nearly parallel to the pH axis between the isoelectric point and a pH value about 9.0. The cation adsorption and exchange capacity by the proteins at neutrality must, therefore, be small as compared to that of the stronger soil acidoids.

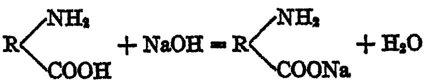
PROTEINATED COMPLEXES

The now generally accepted view is that the proteins contain free amino and carboxyl groups reacting as bases:



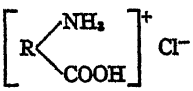
$$R\left\langle\begin{matrix}NH_2\\COOH\end{matrix}\right. + HCl = R\left\langle\begin{matrix}NH_3Cl\\COOH\end{matrix}\right.$$

and as acids:



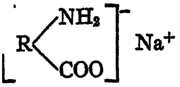
$$R\left\langle\begin{matrix}NH_2\\COOH\end{matrix}\right. + NaOH = R\left\langle\begin{matrix}NH_2\\COONa\end{matrix}\right. + H_2O$$

In the first case a positive protein ion or ion complex will be formed by dissociation thus:



$$\left[R\left\langle\begin{matrix}NH_3\\COOH\end{matrix}\right.\right]^+ Cl^-$$

and in the second case a negative protein ion or ion complex thus:



$$\left[R\genfrac{}{}{0pt}{}{\diagup NH_2}{\diagdown COO}\right]^- Na^+$$

The isoelectric point must be that point at which an equal number of diffusible anions and cations are dissociated by the protein complex. It has been shown (12) that the soil complex contains diffusible anions and cations at the isoelectric point. This proves that the combination with acids and bases overlap, that is, some cations will displace H ions on the acid side and some anions will displace OH ions on the alkaline side of the isoelectric point. The same is probably true of the proteins, in which case the isoelectric condition represents the amphoteric ion $R\genfrac{}{}{0pt}{}{\diagup NH_3^+}{\diagdown COO^-}$. This might be looked upon as an inner salt which is completely ionized analogous to $CH_3\ COO^- + NH_4^+$ as pointed out by Michaelis (13b). But there can be only a small amount of these amphoteric ions because the proteins are very weak acids and bases and therefore extensively hydrolyzed into the molecular species $R\genfrac{}{}{0pt}{}{\diagup NH_2}{\diagdown COOH}$ analogous to the reaction: $NH_4^+ + CH_3\ COO^- \rightleftharpoons NH_3 + CH_3COOH$.

In acid solutions the ionization of the carboxyl group is suppressed while the ionization of the ammonium group is increased. The protein becomes then a positive ion or ion complex. In alkaline solutions or in solutions having a higher pH than that of the isoelectric point the ionization of the ammonium group is suppressed while the ionization of the carboxyl group is increased. The protein is therefore electronegative. As long as the compound formed with the added acid and base are highly ionized the isoelectric point will remain the same.

But if the acid or base (anion or cation) with which the protein combines forms a non-ionized compound then the aforementioned simple rule no longer applies. A new complex will be formed which possesses a different isoelectirc point. Some of these complexes which play an important rôle in the formation and properties of soils will now be discussed.

ALUMINUM AND IRON "PROTEINATES"

The sesquioxides of iron and aluminum were found to be isoelectric at pH values of 7.1 and 8.1 respectively when precipitated from the chlorides by NaOH (9). On the acid side of their isoelectric point the sesquioxides become increasingly electropositive as a result of the dissociation of the compound formed with the acid ion thus:

$$[(Al_2O_3)_{x-1}\cdot Al_2O_3]^{++} + 2Cl^-$$

and

$$[(Al_2O_3)_{x-2}\cdot 2Al_2O_3]^{++++} + 4Cl^- \text{ etc.}$$

The proteins which we have here studied were found to be isoelectric at pH values from 4.8 to 5.2. At higher pH values the proteins become increasingly negative as a result of the dissociation of the proteinate formed with the base (NaOH) thus:

$$[(NH_2)_n \cdot R \cdot (COOH)_{m-1}\ COO]^- + Na^+$$

and

$$[(NH_2)_n \cdot R \cdot (COOH)_{m-2}\ (COO)_2]^{--} + 2Na^+ \text{ etc.}$$

where R represents a protein molecule or molecular aggregate containing n free NH_2 groups and m free COOH groups.

Below a pH of 8.1 alumina exists as a cation while above a pH of 4.8 casein exists as an anion. Now since these ions of opposite charge are non-diffusible colloidal ions we should expect a double displacement to take place, within the pH range 4.8–8.1, upon mixing the two solutions resulting in the formation of aluminum "caseinate" (or more correctly, caseinated alumina) and NaCl. This combination would show itself in a complete or partial electrical neutralization of the colloids. A new complex would be formed having an isoelectric point somewhere between pH 4.8 and 8.1, the exact value depending entirely on the relative proportions of the two components.

Since alumina grows progressively more positive as we decrease the pH from 8.1 to 4.8 and since casein increases in negative strength in the opposite direction it is evident that the proportion of the two components which will yield an isoelectric complex at any particularlar pH will be inversely proportional to their "strength." Near pH 4.8 a little alumina and much casein will be required while near pH 8.1 it will require much alumina and little casein.

The important fact is that every proportion of alumina and casein can be isoelectrically precipitated. This must apply to all mixtures of amphoteric colloids although it has often been stated by several investigators, who have paid no attention to the pH, that such colloids are precipitated in certain proportions only.

In table 75 are shown the flocculation and cataphoresis at various pH values of three aluminum caseinate systems. Space does not allow a more extensive tabulation but the three systems are sufficient to show the law governing the reactions between amphoteric colloids having different isoelectric points.

The results are graphically shown in figure 12. The relationship between composition and the isoelectric pH is more strikingly brought out by including in the figure the curves for "free" alumina on the one side and "free" casein on the other.

It will be noted that a considerable quantity of protein is required to lower the isoelectric pH of alumina while, conversely, small amounts of alumina cause a considerable increase in the isoelectric pH of the protein. This is, of course, in the first place, due to the high equivalent weight of the protein and, in the second place, to the weakness of the protein as an acid so near its isoelectric point. With 5 millimols $AlCl_3$ to 1 gm. casein the isoelectric point is at pH 7.4 and with as little as 1/3 millimol $AlCl_3$ (about 0.017 gm. Al_2O_3 to a gm. of casein) the complex is isoelectric at pH 5.5. The actual composition of the isoelectric complexes is given in table 81a, and the relation between the isoelectric composition and the pH is shown graphically in figure 14, both of which will be discussed later.

TABLE 75

The $AlCl_3$ + Na —Caseinate systems

SOLUTION A, 20 cc. + HCl*	SOLUTION B, 20 cc. + NaOH*	FLOCCULATION		μ/SEC. 1 VOLT/CM.	pH
		After mixing	Overnight		
millimol	*millimol*				
No. 121 A: 5 millimols $AlCl_3$ in 1,000 cc. B: 1 gm. casein + 1 millimol NaOH in 1,000 cc.					
0.0	0.25	Opal	0	......	6.2
0.0	0.26	Instant	++++	+1.21	6.6
0.0	0.27	Instant	++++	±0.0	7.4
0.0	0.275	Instant	++++	−0.34	7.6
0.0	0.28	Instant	++++	−0.92	7.9
0.0	0.30	Opal	0	......	8.8
No. 121 A: 1 millimol $AlCl_3$ in 1,000 cc. B: 1 gm. casein + 1 millimol NaOH in 1,000 cc.					
0.0	0.0325	Opal	++	+1.35	6.0
0.0	0.035	Opal	++++	+0.55	6.3
0.0	0.0375	Rapid	++++	+0.17	6.55
0.0	0.040	Instant	++++	−0.40	6.7
0.0	0.0425	Slow	++	−0.98	6.8
0.0	0.050	Opal	0	......	7.15
No. 123 A: ⅓ millimol $AlCl_3$ in 1,000 cc. B: 1 gm. casein + 1 millimol NaOH in 1,000 cc.					
0.015	0.0	Opal	0	......	4.8
0.010	0.0	Slow	++++	+0.61	5.2
0.0075	0.0	Instant	++++	±0.0	5.5
0.005	0.0	Instant	++++	−0.67	5.75
0.0025	0.0	Rapid	+++	−0.87	5.9
0.0	0.0	Turbid	++	−1.08	6.2
0.0	0.005	Opal	0	......	6.6

* Plus enough water to make 50 cc.

Compared to the humate complex the proteins are therefore much weaker as acids and as electronegative colloids. One millimol of $AlCl_3$ to 1.05 gm.

humus resulted in a complex which was electronegative at all pH values, whereas 2 millimols $AlCl_3$ to the same amount of humus yielded an isoelectric complex at pH about 4.5 (10).

Experiments with $FeCl_3$ instead of $AlCl_3$ have shown that, as in the case of the silicated, phosphated, and humated complexes, the proteinated ferric complexes are isoelectric at a lower pH than the corresponding Al-complexes.

Albumin, edestin, and gelatin were studied in a single proportion with $AlCl_3$, namely in the ratio of 1 millimol $AlCl_3$ to 1gm. protein. The results are shown in table 76.

It will be noted that both edestin and gelatin, which in the "free" condition are isoelectric at a higher pH than either casein or albumin, and are therefore more weakly acidic, suppress the isoelectric pH of their aluminum complexes less than the latter proteins. Such differences in the relative strength of the acid and basic groups of the proteins express themselves consistently, especially in the case of edestin and casein, which have been subjected to a comparative study (see figure 13). Gelatin has been found to behave somewhat erratically, probably because of hydrolysis which this protein rapidly undergoes in spite of the fact that the solutions were kept only a short time and that microbial activity was inhibited by the addition of toluene.

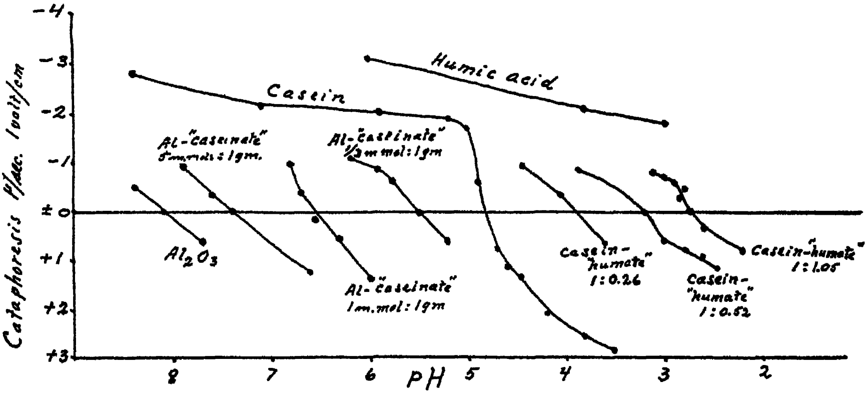


FIG. 12. CATAPHORESIS AND ISOELECTRIC POINTS OF A SERIES OF AL-"CASEINATES" AND OF CASEIN-"HUMATES"

The property of gelatin of not coagulating at its isoelectric point reflects itself in the Al-"gelatinate." A mere opalescence but no flocculation was observed in any of the tubes after mixing. But on the following day flocculation was complete near the isoelectric point. The figures in table 81A show, however, that only about 25 per cent of the gelatin was carried down as compared to about 90 per cent of the other proteins. This lack of reactivity of the gelatin, which has already been referred to, expresses itself in all the complexes studied.

The question as to the nature of the compounds formed in the preceding reactions will be discussed later. It is desirable first to discuss another type of proteinated complexes.

THE PROTEIN "HUMATES"

In the preceding chapter we have studied the behavior of proteins as negative colloids, as acids, and as anions. We shall now bring the same proteins below their isoelectric pH and study them as positive colloids, as bases, and as cations. In this condition we shall find them to combine with negative colloids, with acids, and with anions. If the substance we introduce into a solution of a positive protein yields anions or anion complexes, which form

TABLE 76

The $AlCl_3$ + Na-albuminate, Na-edestinate, and Na-gelatinate systems

SOLUTION A	SOLUTION B, 20 cc. + NaOH	FLOCCULATION		μ/SEC. 1 VOLT/CM.	pH
		After mixing	Overnight		
cc.	*millimol*				
		No. 125			
A: 1 millimol $AlCl_3$ in 1,000 cc.					
B: 1 gm. albumen + 1 millimol NaOH in 1,000 cc.					
20	0.030	Opal	0	......	5.8
20	0.035	Instant	++++	+0.15	6.1
20	0.0375	Instant	++++	−0.42	6.4
20	0.040	Rapid	+++	−0.72	6.6
20	0 0425	Opal	0	.....	6.8
		No. 124			
A: 1 millimol $AlCl_3$ in 1,000 cc.					
B: 1 gm. edestin + 1 millimol NaOH in 1,000 cc.					
20	0.040	Opal	0	......	6 3
20	0.045	Slow	++++	+0.92	6.65
20	0 0475	Instant	++++	+0.47	7.0
20	0.050	Instant	++++	±0.0	7.15
20	0.0525	Slow	++++	−0.45	7.4
20	0.060	Opal	+	−1 01	7.7
		No. 126			
A: 1 millimol $AlCl_3$ in 1,000 cc.					
B: 1 gm. gelatin + 1 millimol NaOH in 1,000 cc.					
20	0.050	Clear	0	......	6.9
20	0.0525	Opal	++	+0.40	7.0
20	0.055	Opal	++++	+0.18	7.15
20	0.0575	Opal	++++	− slight	7.6
20	0.060	Opal	+++	−0.26	7.8
20	0.065	Clear	++	−0.76	8.2

with the protein a non-ionized complex, then we shall find that the isoelectric point of this complex is lower on the pH scale than that of the original protein.

TABLE 77

The casein-"humate" systems

SOLUTION A, 20 cc. + HCl	SOLUTION B	FLOCCULATION		μ/SEC. 1 VOLT/CM.	pH
		After mixing	Overnight		
millimol	*cc.*				
No. 131 A: 0.5 gm. casein + 0.5 millimol NaOH in 1,000 cc. B: 1.5 m. equiv. Na-humate in 1,000 cc.					
0.04	20	Opal	0	......	....
0.06	20	Slow	++++	−0.82	3.1
0.08	20	Instant	++++	−0.61	2.9
0.10	20	Instant	++++	−0.32	2.85
0.13	20	Instant	++++	±0.0	2.75
0.18	20	Instant	++++	+0.30	2.6
0.28	20	Instant	++++	+0.76	2.2
No. 132 A: 1 gm. casein + 1 millimol NaOH in 1,000 cc. B: 1.5 m. equiv. Na-humate in 1,000 cc.					
0.04	20	Opal	0	−1.78	....
0.06	20	Rapid	++++	−0.87	3.9
0.08	20	Instant	++++	±0.0	3.2
0.10	20	Instant	++++	+0.61	3.0
0.14	20	Instant	++++	+0.76	2.8
0.18	20	Slow	++++	+0.90	2.6
0.28	20	Opal	++	+1.16	....
No. 133 A: 1 gm. casein + 1 millimol NaOH in 1,000 cc. B: 0.75 m. equiv. Na-humate in 1,000 cc.					
0.04	20	Opal	0	......	....
0.045	20	Rapid	++++	−0.93	4.45
0.05	20	Instant	++++	−0.34	4.05
0.06	20	Instant	++++	+0.64	3.6
0.08	20	Slow	++	+1.11	....
0.10	20	Turbid	+	+1.17	....

For the study of such complexes in which the protein plays the part of the basic group we have selected the following soil acidoids: humic acid from peat, bentonite, and silicic acid.

The humic acid was employed in the form of Na-"humate" containing 0.35 gram dry weight humus to each millimol NaOH. The same preparation has been described in section IV of this series. The flocculation and cataphoresis at various pH values of three different mixtures of casein and Na-humate are shown in table 77. Table 78 shows the same relationship with albumin, edestin, and gelatin in a single proportion of each with the humate.

TABLE 78

The albumin-edestin- and gelatin-"humate" systems

SOLUTION A, 20 cc. + HCl	SOLUTION B	FLOCCULATION		μ/SEC. 1 VOLT/CM.	pH
		After mixing	Overnight		
millimol	*cc.*				
No. 135 A: 1 gm. albumin + 1 millimol NaOH in 1,000 cc. B: 1.5 m. equiv. Na-humate in 1,000 cc.					
0.06	20	Opal	0	−1.44	....
0.065	20	Rapid	++++	−0.87	4.0
0.07	20	Instant	++++	−0.52	3.8
0.08	20	Instant	++++	±0.0	3.4
0.09	20	Instant	++++	+0.52	3.25
0.10	20	Slow	++++	+0.70	3.15
0.12	20	Turbid	+++	+0.95	3.0
No. 134 A: 1 gm. edestin + 1 millimol NaOH in 1,000 cc. B: 1.5 m. equiv. Na-humate in 1,000 cc.					
0.05	20	Opal	+	−1.51	....
0.055	20	Instant	++++	−1.26	4.55
0.06	20	Instant	++++	−0.47	4.1
0.07	20	Instant	++++	+0.46	3.5
0.08	20	Rapid	++++	+0.78	3.35
0.09	20	Rapid	++++	+1.01	3.25
0.10	20	Turbid	+++	+1.21	3.15
No. 136 A: 1 gm. gelatin (no NaOH) in 1,000 cc. B: 1.5 m. equiv. Na-humate in 1,000 cc.					
0.03	20	Opal	0	......	....
0.035	20	Instant	++++	−slight	4.2
0.04	20	Instant	++++	+slight	3.9
0.045	20	Instant	++++	+0.43	3.7
0.05	20	Slow	+++	+0.50	3.5
0.06	20	Opal	+	+0.90	....

In the aluminum "proteinate" systems we were dealing with reactions between two amphoteric components. The isoelectric point of any complex of these two components will be found between the isoelectric points of the protein and of the alumina. In the protein-"humate" systems we are dealing with the reactions between an amphoteric component on the one side and a strictly electronegative and acidic component on the other. The result of this reaction will be as follows: As the humus or the negative component is increased the isoelectric point will be displaced more and more to the acid side until, with a certain excess of the humus or negative component, the resulting complex is no longer electrokinetically amphoteric but migrates anodically at all pH values.

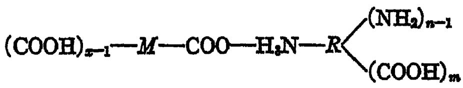
The protein-"humate" systems resemble in behavior the aluminum and ferric "humates" which were discussed in previous sections (10), but the isoelectric points of the former are, of course, much lower because of the weaker basic properties of the proteins. The relationship is best seen in figures 8 and 12. In figure 12 the cataphoresis of humic acid in HCl solution is also shown. In system no. 131, in which the proportion of humate to casein is the highest, we note that the positive charge is low and that the complex is completely flocculated at a pH as low as 2.2. When the proportion of humus exceeds a certain limit the complex must remain negative and approach in behavior that of "free" humus.

If we assume the "humic acid" to be strictly acidic [which is not true because all humus contains some nitrogen, indicating proteins, and although the peat humus here employed is cataphoretically negative at all pH values its amphoteric reaction was demonstrated by an exchange alkalinity (12) at low pH values], we might represent it by a general formula if we make the further assumption that its acidic property is due to COOH groups thus: $M(\mathrm{COOH})_x$ and its sodium compounds thus:

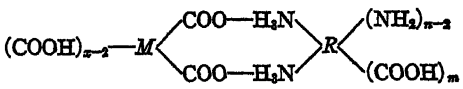
$$[M\cdot(\mathrm{COOH})_{x-1}\cdot\mathrm{COO}]^{-}+\mathrm{Na}^{+}$$

$$[M\cdot(\mathrm{COOH})_{x-2}\cdot2\mathrm{COO}]^{--}+2\mathrm{Na}^{+}$$

etc., in which M represent the molecule or molecular aggregate to which x groups of COOH are linked. The complexes which the positive proteins form with humic acids may then be expressed by the formulas:



$$(\mathrm{COOH})_{x-1}\text{—}M\text{—COO—H}_3\text{N—}R\left\langle\begin{matrix}(\mathrm{NH}_2)_{n-1}\\(\mathrm{COOH})_m\end{matrix}\right.$$



$$(\mathrm{COOH})_{x-2}\text{—}M\left\langle\begin{matrix}\text{COO—H}_3\text{N}\\\text{COO—H}_3\text{N}\end{matrix}\right\rangle R\left\langle\begin{matrix}(\mathrm{NH}_2)_{n-2}\\(\mathrm{COOH})_m\end{matrix}\right.$$

etc.

These complexes would react amphoterically and become positively charged in solutions sufficiently acid to yield a greater number of the cationic species, $[R-\mathrm{NH}_3]^{+}$ than of the anionic species, $[R-\mathrm{COO}]^{-}$.

The pH at which the two forms of dissociation balance each other must be the isoelectric pH. Above this pH the complex would be negative because of preponderance of the anionic species. A complex in which the non-diffusible acidic component (e.g. the humus) exceeds the non-diffusible basic component (e.g. the protein) must remain negative at all pH values either because all the basic groups are linked up in a non-ionizing combination thus:

$$(\mathrm{COOH})_{x-n}—M—(\mathrm{COO})_n—(\mathrm{H_3N})_n—R—(\mathrm{COOH})_m$$

or because the number of the NH_2 groups, free to combine with the diffusible acid (e.g. HCl), is insufficient to form the required excess of the cationic species. In other words the anionic dissociation by the complex would never exceed the cationic dissociation at any pH.

It is interesting to note that gelatin, which, when alone, does not flocculate even at its isoelectric point, is instantly flocculated together with humic acid. The amount of gelatin carried down in the floc is, however, in every case less than that of any other of the proteins (see table 81b). When we speak about gelatin or any other protein as a "protective" colloid we should be careful to define the conditions and keep in mind the chemical nature of the substance to be "protected."

THE PROTEIN "BENTONATES"

It is realized that the term "bentonate" is a very loose one but we shall find it expedient to use it just as, and for the same reason that, we have found it expedient to employ the term "humate." Bentonite is distinctly acidic and gives no indications of being amphoteric either cataphoretically or in its exchange reactions (12). Electrodialyzed, its suspension was found to be more acid (pH 2.6) than that of the aforementioned sample of humus but the cation exchange capacity of the bentonite at pH 7.0 is only a little more than one-third of that of the humus, or 1.02 and 2.87 milliequivalents per gram respectively. At a pH of 2.0 the bentonite adsorbed, however, considerable Ca ions (12) whereas the Ca-ion adsorption by humus reached a vanishing point at this pH. Bentonite must therefore be looked upon as a stronger acid than humus. This can partly be ascribed to the presence of proteins in the latter. The equivalent weight of the humus is, however, about one-third that of bentonite and possesses therefore the greatest neutralizing power. All this is mentioned because of its relation to the way these acidoids react with the proteins.

Table 79 shows the flocculation and cataphoresis at various pH values of casein and edestin "bentonates." Albumin and gelatin were also included but except for the composition of the isoelectric floc (table 81c) the tabulation is omitted. The bentonite was used in the highly dispersed Na-saturated condition.

All the proteins flocculate isoelectrically with bentonite at a pH below that of the "free" proteins. But the displacement of the isoelectric point is not nearly as great as in the case of the "humates." With a ratio of 1:1 of casein to bentonite the isoelectric point is displaced from about 4.8 to about 4.25

(interpolated). With the same ratio of edestin to bentonite the isoelectric point was displaced from 5.2 to about 4.6. With a ratio of 2 of protein to 1.05 of humus the isoelectric points were displaced to pH 3.2 in the casein and 3.8 in the edestin system. With a ratio of 1 casein to 1.05 humus the system was isoelectric as low as pH 2.75.

The humus with nearly three times the neutralizing power per gram as compared to bentonite, causes by far the greatest displacement of the isoelectric point of the proteins to the acid side. This speaks strongly in favor of an actual combination between the basic group in the proteins and the acid group of the humus and bentonite as suggested in the foregoing. A more direct proof of such combination will be given later.

TABLE 79

The casein- and edestin-"bentonate" systems

SOLUTION A, 20 CC. + HCl	SOLUTION B	FLOCCULATION		μ/SEC. 1 VOLT/CM	pH
		After mixing	Overnight		
millimol	*cc.*				
No. 111 A: 1 gm. casein + 1 millimol NaOH in 1,000 cc. B: 1 gm. bentonite in 1,000 cc.					
0.035	20	Opal	0	+1.89	....
0.040	20	Instant	+++	−1.68	5.6
0.045	20	Instant	++++	−1.38	5.5
0.050	20	Instant	++++	−0.25	4.4
0.060	20	Instant	++++	+0.70	3.9
0.080	20	Rapid	++	+1.48	....
0.120	20	Slow	+	+1.65	....
No. 112 A: 1 gm. edestin + 1 millimol NaOH in 1,000 cc. B: 1 gm. bentonite in 1,000 cc.					
0.025	20	Turbid	0	−1.78 (?)	....
0.030	20	Instant	++	−1.89 (?)	5.9
0.035	20	Instant	+++	−1.55	5.7
0.040	20	Instant	++++	−1.10	5.3
0.045	20	Instant	++++	−0.30	4.65
0.050	20	Rapid	+++	+1.12	4.4
0.060	20	Slow	++	+1.89	4.35
0.120	20	Turbid	0	+1.89	....

THE PROTEIN SILICATES

Silicic acid is an extremely weak acid and adsorbs only small amounts of cations from neutral salt solutions. Its cation exchange capacity is therefore very small as compared to the hydrous alumino-silicates such as bentonite and the synthetic silicates discussed in the foregoing sections (11). Why silicic acid should be a stronger acid in (partial) combination with Al and Fe than in the free state has never been explained. The structure might be a factor, as previously suggested (11), but it might be that silicic acid is a pseudo-acid existing in water largely as SiO_2 and only to a small extent as H_2SiO_3 or H_4SiO_4. In partial combination with the hydroxides of Al and Fe thus:

$$(OH)_2\text{—Al—SiO}_3\text{—H}$$

TABLE 80

The casein and edestin "silicate" systems

SOLUTION A, 20 cc. + HCl	SOLUTION B	FLOCCULATION		μ/SEC. 1 VOLT/CM.	pH
		After mixing	Overnight		
millimol	*cc.*				
No. 115 A: 1 gm. casein + 1 millimol NaOH in 1,000 cc. B: 10 millimols Na_2SiO_3 in 1,000 cc.					
0.460	20	Opal	0	......	5.35
0.465	20	Slow	++++	−0.73	4.9
0.467	20	Rapid	++++	±0.0	4.7
0.470	20	Rapid	++++	+0.47	4.5
0.475	20	Opal	+	......	4.35
0.48	20	Opal	0	......	4.25
No. 116 A: 1 gm. edestin + millimol NaOH in 1,000 cc. B: 10 millimols Na_2SiO_3 in 1,000 cc.					
0.440	20	Opal	0	......	6.3
0.450	20	Milky	+	......	5.85
0.455	20	Instant	++++	−1.50	5.65
0.4575	20	Instant	++++	−0.76	5.4
0.460	20	Instant	++++	−0.43	5.25
0.465	20	Milky	+++	+1.01	5.0
0.47	20	Opal	0	......	4.8

a reversion to the SiO_2 could not take place thus leaving a stabilized acid residue. This problem is now being investigated in the form of a series of alumino-silicates of great variations in composition.

The weakness of silicic acid expresses itself interestingly in its reaction with the proteins. The results with casein and edestin are shown in table 80. Albumin reacted in a similar manner but gelatin, which flocculated more or less hesitatingly in the preceding systems, was entirely unaffected by silicic acid as far as the optical appearance went.

The weak silicic acid which adsorbs only small amounts of the common cations from neutral solutions combines evidently very feebly with the protein cations at the low pH at which these substances become cationic. The isoelectric point of the proteins is displaced to the acid side in a barely perceptible degree by as much as 10 millimols of silicic acid per gram protein.

The displacement is best seen in figure 13, in which the various casein and edestin systems are compared. The comparison is instructive because it shows fairly constant differences in the isoelectric points of the two series of proteinated

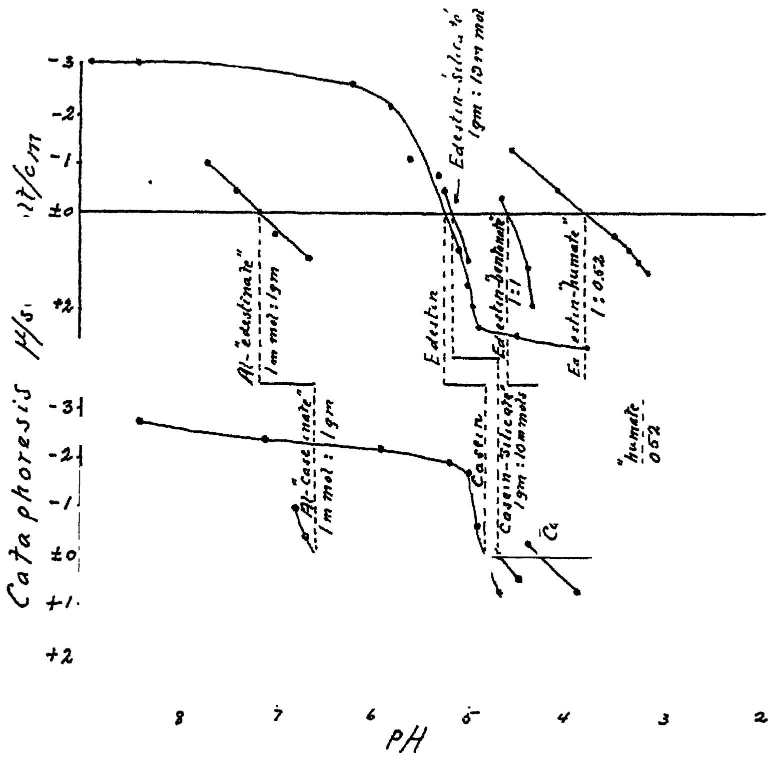


FIG. 13. THE ISOELECTRIC POINTS OF CASEIN AND EDESTIN AND SOME OF THEIR COMPOUNDS, COMPARED

complexes. The broken line perpendiculars indicate the differences of the two systems. The isoelectric points of "free" edestin and casein differ by about 0.4 pH. This order of magnitude is, within certain limits, maintained throughout the two series.

This all speaks well in favor of the accuracy of the method here employed in measuring the cataphoretic movement of ultramicroscopic particles. The cell constructed by the author has the great advantage of enabling the experimenter to make a great number of measurements in a short time. Mass work of this kind is, however, unavoidably done at the expense of great accuracy. Greater accuracy may be attained but only at the expense of much time. The sources of errors in the foregoing measurements are not entirely cataphoretic but of the most intricate and varied origin. Thus besides errors in pH the systems are affected by the mode of mixing the colloids, but the drift in the magnitude of the charge and of the isoelectric point, by hydrolysis, by the temperature, etc. The systems are dynamic and unstable, the colloidal complex, especially the amphoteric complex, is very sensitive.

THE COMPOSITION OF THE ISOELECTRIC COMPLEXES

The composition of the various isoelectric complexes was determined as follows: Using 0.2 gm. of protein, the various systems, containing the proportions studied cataphoretically, were isoelectrically precipitated by adding the calculated amount of HCl or NaOH. The floc was filtered off but not washed, dried in the air, and, together with the paper, digested in Kjeldahl flasks. The nitrogen in the original proteins, in the various precipitates, in the humus, in the bentonite, and in the paper was determined and on this basis the quantities of the proteins carried down in the floc were calculated. The SiO_2 was determined in the supernatant liquid, the difference being accounted to the floc. The humus, the bentonite, and the sesquioxides were quantitatively precipitated in all of the systems studied. The results are shown in tables 81 a, b, and c. The composition of the various Al-"caseinates" and casein-"humates" are plotted as ordinates against the isoelectric pH values as abscissas in figure 14. The curves, representing the two series of compounds, originate on the abscissa axis, at pH 4.8, the isoelectric point of "free" casein.

If we examine the various Al-"caseinates" and casein "humates" we find that the percentage of casein precipitated is somewhat higher, the higher the proportion of aluminum or humus. It is also noteworthy that of the 10 millimols of SiO_2 added to 1 gm. each of casein and edestin only 0.84 millimol and 0.63 millimol respectively were precipitated. The percentage of gelatin which precipitated is smaller than that of any of the other proteins. Gelatin and silicic acid did not, under the conditions of the experiment, form any precipitate although such a percipitation is recorded by Graham, who worked with more concentrated solutions (1).

We note that in no case is the protein quantitatively precipitated. Whether the precipitated and non-precipitated fractions of the proteins are identical or of a different nature has not been determined. It is conceivable that the fraction which remains in solution represents, on the one side a basic group displaced by Al and Fe and on the other side an acid group displaced by the humus and bentonite.

The so-called "simple" proteins may constitute compounds of proteins having different isoelectric points just as the "conjugated" proteins are compounds of proteins with non-protein groups. Thus the basic histones would undoubtedly combine with the acidic prolamines forming complexes which would all be

TABLE 81A

The composition and pH of isoelectric Al- and Fe-"proteinates"

(Dilution = 1 gm. protein in 2,500 cc.)

SYSTEM NUMBER	COMPLEX	COMPOSITION RATIO OF SYSTEM		ISOELECTRIC pH	PROTEIN PRECIPITATED	COMPOSITION OF ISOELECTRIC COMPLEX	
		R_2O_3	Protein			R_2O_3	Protein
		millimol	*gm.*		*per cent*	*millimol*	*gm.*
127	Fe-caseinate	1	0.40	7.0	92.8	1	0.37
121	Al-caseinate	1	0.40	7.4	89.2	1	0.36
123	Al-caseinate	1	6.00	5.5	81.5	1	4.89
122	Al-caseinate	1	2.00	6.6	84.2	1	1.68
124	Al-edestinate	1	2.00	7.15	90.5	1	1.81
125	Al-albuminate	1	2.00	6.2	92.5	1	1.85
126	Al-gelatinate	1	2.00	7.4	25.4	1	0.51

TABLE 81B

The composition and pH of isoelectric protein "humates"

SYSTEM NUMBER	COMPLEX	COMPOSITION RATIO OF SYSTEM		ISO-ELECTRIC pH	PROTEIN PRECIPITATED	COMPOSITION OF ISOELECTRIC COMPLEX	
		Protein	Humus			Protein	Humus
		gm.	*gm.*		*per cent*	*gm.*	*gm.*
131	Casein-humate	1.00	1.05	2.75	95.0	0.905	1.00
133	Casein-humate	1.00	0.262	3.9	89.9	3.43	1.00
132	Casein-humate	1.00	0.525	3.2	93.6	1.78	1.00
134	Edestin-humate	1.00	0.525	3.8	94.7	1.80	1.00
135	Albumin-humate	1.00	0.525	3.4	94.7	1.80	1.00
136	Gelatin-humate	1.00	0.525	4.0	70.2	1.34	1.00

TABLE 81C

The composition and pH of isoelectric protein-"bentonates" and "silicates"

SYSTEM NUMBER	COMPLEX	COMPOSITION RATIO OF SYSTEM		ISO-ELECTRIC pH	PROTEIN PRECIPITATED	COMPOSITION OF ISOELECTRIC COMPLEX	
		Protein	Bentonite			Protein	Bentonite
		gm.	*gm.*		*per cent*	*gm.*	*gm.*
111	Casein-bentonate	1.00	1.00	4.25	95.1	0.951	1.00
112	Edestin-bentonate	1.00	1.00	4.58	94.5	0.945	1.00
113	Albumin-bentonate	1.00	1.00	4.20	97.7	0.977	1.00
			SiO_2			SiO_2 PRECIPITATED	
			millimols			*per cent*	
115	Casein-silicate	1.00	10.00	4.7	79.5	8.4	
116	Edestin-silicate	1.00	10.00	5.15	82.0	6.3	

isoelectric at pH values between those of the "simple" proteins. Such combination must result within the animal and plant organism. For this reason no two samples of any one kind of protein can be expected to be exactly alike. It might be said about the proteins that they are products of their environment.

The chemistry of the proteins which, at the time of Emil Fischer, looked so promising has made very little progress and, in the opinion of as eminent an authority as Levene (3), promises little for the future. But where the methods which have proved so useful in the organic chemistry of the more simple compounds fail, the methods of colloid chemistry might be successfully applied.

THE NATURE OF THE COMPOUNDS

The most important question arising from the preceding data is the mechanism by which the various components react to form new complexes. The problem of the mutural flocculation of oppositely charged colloids has been extensively investigated and many of the investigators are satisfied with the explanation that the colloid, being stabilized by its charge, must flocculate when this charge is neutralized. Some others (15) would explain the phenomenom by assuming that the charge and stability of the colloid are due to an extraneous stabilizing electrolyte and that the electrical neutralization and flocculation is the result of a chemical reaction between the stabilizing electrolytes. By what valences or forces the stabilizing electrolyte is attracted to the colloid in the first place and what satisfies these valences after the stabilizer is removed are not considered. The colloids themselves do not react, they are not ionogens but merely carriers of ions.

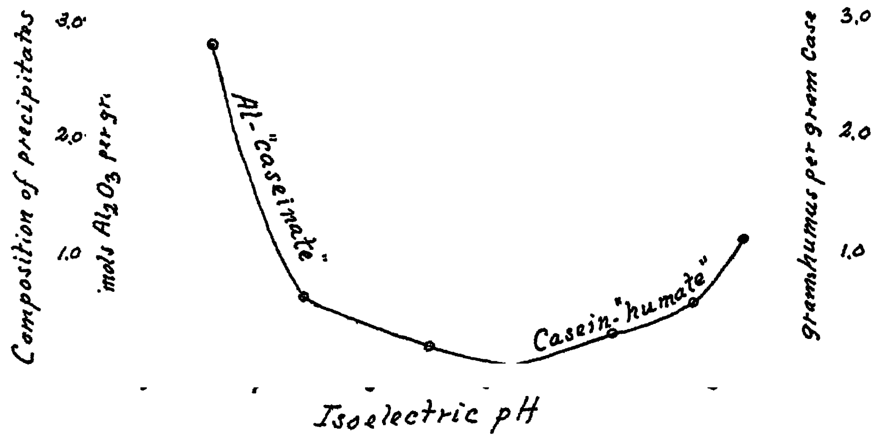


FIG. 14. RELATION BETWEEN COMPOSITION AND ISOELECTRIC pH OF Al-"CASEINATE" AND CASEIN-"HUMATE"

Although this explanation may be true of inert substances such as paraffin, collodion, and air bubbles, it does not fit the case of reactive colloids like the acidoids, ampholytoids, and their compounds. We might as well say that

NaOH stabilizes CO_2, that HCl stabilizes CaO, and that, when the solutions are mixed, a precipitate is formed because the stabilizers react, as to say that NaOH stabilizes a protein, that HCl (or $AlCl_3$) stabilizes alumina, and that, when the two are mixed, flocculation occurs because a chemical reaction removes the stabilizers. We would be stating the most unessential half of the truth, for we would have said nothing about calcium carbonate and nothing about the complex of alumina and protein which was formed by the double displacement.

If alumina, or rather aluminum oxychloride, is positive thus:

$$[(Al_2O_3)_x—Al_2O_2]^{++}\ 2Cl^-$$

TABLE 82

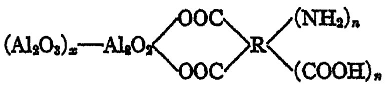
The adsorption of SO_4 ions by alumina and aluminum-"albuminate" at various pH

A's: 0.5 millimol $Al_2(SO_4)_3$ in 250 cc.
B's: 0.5 millimol $Al_2(SO_4)_3$ + 1 gm. albumin in 250 cc.

SOLUTION	NaOH	pH	SO_4		Al_2O_3	
			In solution	In precipitate	In solution	In precipitate
	millimols		*millimols*	*millimol*	*millimol*	*millimols*
A1	1.75	4.25	1.26	0.24	0.122	0.378
A2	2.00	4.4	1.24	0.26	0.075	0.425
A3	2.50	6.45	1.31	0.19	None	0.500
B1	1.75	4.7	1.27	0.23	0.059	0.441
B2	2.00	5.0	1.285	0.215	0.018	0.482
B3	2.25	5.9	1 35	0.15	None	0.500

Composition of precipitates:
A1: $Al_2O_3\cdot(SO_3)_{0.635}$ B1: $Al_2O_3\cdot(SO_3)_{0.516}$
A2: $Al_2O_3\cdot(SO_3)_{0.618}$ B2: $Al_2O_3\cdot(SO_3)_{0.447}$
A3: $Al_2O_3\cdot(SO_3)_{0.375}$ B3: $Al_2O_3\cdot(SO_3)_{0.305}$

and is rendered isoelectric by the removal of the dissociated Cl ions then some other anions which do not dissociate must take their place. In the case of the Al-"proteinates" it is assumed that the protein anions have displaced the diffusible Cl ions thus:



Now this complex can combine with more Cl at a lower pH and again become positive, but at a given pH the proteinated complex should contain less Cl than the free alumina, provided the pH is not so low that the amino groups combine with appreciable quantities of HCl. To verify this the pH must therefore be kept well above the isoelectric point of the protein.

This has been investigated, but instead of the Cl ion the SO_4 ion was selected because the latter ion is more strongly adsorbed by alumina and, what is more important, this divalent anion keeps the positive complex flocculated over a much greater range. Aluminum sulfate was therefore used instead of the chloride. Albumin was used because this protein was free from both calcium and sulfates. The results are shown in table 82.

The experiment shows clearly that the protein, reacting as anions, displaces the SO_4 ions just as we found the silicate ions to do in the silicated complexes (9). Since the pH values of the A and B solutions are not the same, the relationship is best brought out in figure 15. It will be noted that the "albuminate" curve is such that if extended on the acid side it would cross the free alumina curve. This is what we should expect, because at lower pH values the amino group becomes active and adsorbs anions.

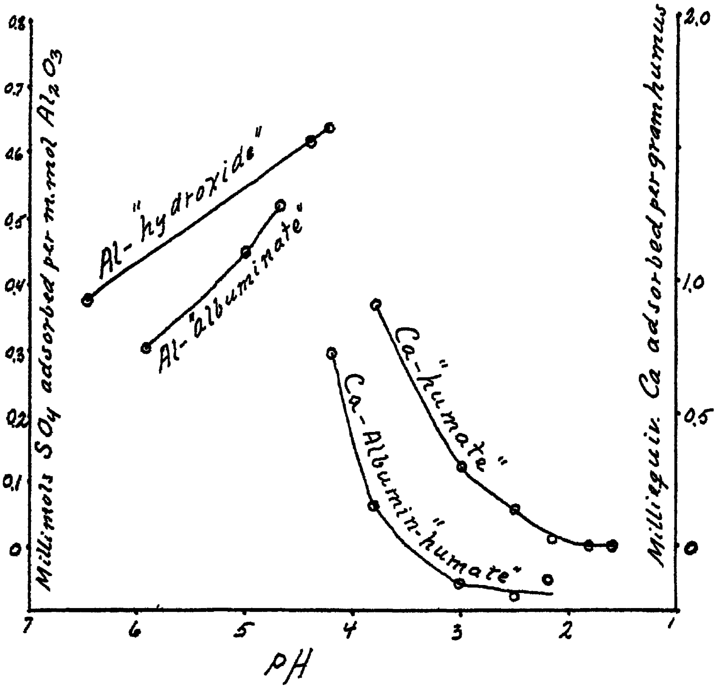


FIG. 15. ALUMINUM "HYDROXIDE" COMBINES WITH (ADSORBS) MORE SO_4 THAN Al-ALBUMINATE (LEFT); HUMIC ACID ADSORBS MORE CA THAN ALBUMIN "HUMATE" (RIGHT)

This proves that protein anions and cations displace the common anions and cations respectively.

We shall now see that the protein cations displace the common cations in combination with acidoids. If the proteins react as cations on the acid side of their isoelectric point and combine as such with the acidoid group, i.e., humus, bentonite, etc. then at a given (low) pH the acidoids should adsorb more of the common cations when alone than when in the presence of proteins, that is, Ca-"humate" should contain more Ca at a pH of 3 or 4 than "Ca-albumin-humate."

To verify this the experiment shown in table 83 was performed. Allowance for the volume occupied by the solids was made by assuming a specific gravity of 1.4 for the organic matter.

At the same pH considerably more Ca was adsorbed by the free humus than by the mixture of albumin and humus. The results are best shown in figure 15. Here again the two curves show a tendency to cross and undoubtedly would cross at higher pH values where the protein is anodic and combines with cations. Since the systems do not flocculate except within the range covered, this could not be verified.

The negative adsorption in two of the albumin-"humate" systems is due to the Donnan distribution of the free ions. The swarm of Cl ions surrounding the positive complexes forces an excess of the free $CaCl_2$ into the external solution. This negative adsorption must be present in all of the other systems as well, except (theoretically) in the isoelectric, but is here more than balanced by the positive adsorption (7).

TABLE 83

The adsorption of Ca ions by humus and by albumin-"humate" at various pH

A's: 3 m. equiv. Na-humate (= 1.05 gm. humus) + 4 m. equiv. $CaCl_2$ in 500 cc.
B's: 3 m. equiv. Na-humate + 2 gm. albumin + 4 m. equiv. $CaCl_2$ in 500 cc.

SOLUTION	HCl	pH	Ca IN SOLUTION	Ca ABSORBED
	m. equiv		*m. equiv.*	*m. equiv.*
A1	2.0	3.8	3.07	0.93
A2	4.0	3.0	3.70	0.30
A3	6.0	2.5	3.86	0.14
A4	8.0	2.15	3.97	0.03
A5	12.0	1.8	4.00	0.00
B1	3.0	4.2	3.27	0.73
B2	4.0	3.8	3.85	0.15
B3	6.0	3.0	4.14	−0.14
B4	10.0	2.5	4.19	−0.19

STOICHIOMETRY

The ion-complexes or micelles of the preceding compounds are highly multivalent, running perhaps in the larger ultramicrons, into the hundreds of thousands (8). The number of combinations is infinite. As previously pointed out, there can therefore be no stoichiometrical relationship between the constituent parts of so complex substances. But is not such a relationship an exception rather than a rule even in the case of "simple" compounds? Within the crystal lattice the law of definite proportions of any pair of ions doubtless holds, but in the solution and at the surface of crystals or at the interface of colloids the composition of compounds may be as complex as the solution itself. We know that ions combine in some way with the water molecules (hydration) but we do not know in what proportions. We know that the ions in solution mutually affect one another but we know very little about the form in which they exist. Modern chemists frequently disagree as to the definition of acids, bases, and salts (16).

The mass law has been found to be only an approximation. The pH of a solution does not express the mass or concentration of the H ions but instead the activity of these ions. The energy or activity concept has taken the place of the mass concept in chemical reactions, and the law of definite and multiple proportions, being essentially a mass law, must likewise be modified by the newer concepts of energetics.

But, it might be argued, the stoichiometric relationship of "pure" compounds is the basis of all methods of analysis. The answer to this is, in the first place, that in the development of analytical methods every conceivable procedure is tried and only that one which yields the comparatively best results is adopted. The result is, in general, a method which is limited to a very narrow set of conditions. We are cautioned against the "occlusion" of this or that ion. In the second place the accuracy of the final determination of the separated "pure" compound is subject to inherent limitations. Thus the SO_4 or Ba ions are measured in terms of barium sulfate, which is always expressed by the formula $BaSO_4$. In other words the barium sulfate precipitate, whatever it is, is used as its own yardstick; we are determining the accuracy of the yardstick by itself.

THE REACTION WITH SILVER CHLORIDE

Silver chloride must be looked upon as one of the most simple compounds. It plays an important rôle in qualitative as well as in quantitative chemistry and respresents, to the analytical chemist, something very definite. Yet the complexity of this substance is easily demonstrated. It probably plays a minor rôle among the soil colloids but from what we have learned during the last years about the rarer elements any positive statement would be premature. But because of its bearing on the subject here studied a brief discussion will be given.

If an excess of NaCl is added to a dilute solution of $AgNO_3$ an opalescent, negative sol is formed. With an excess of $AgNO_3$ an opalescent, positive sol results. In equivalent proportions an isoelectric or nearly isoelectric precipitate is formed. This behavior, characteristic of the silver halides in general, has been extensively investigated by Lottermoser (4).

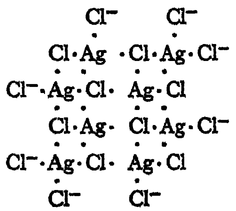
If in a series of beakers we add an increasing excess of NaCl in one direction and an increasing excess of $AgNO_3$ in the other and examine the sols thus formed we find that the dispersibility increases, as shown by a decreasing opalescence, as the excess of either reagent is increased. If we examine the various sols cataphoretically we find that the negative charge increases progessively with an increase of NaCl whereas the positive charge increases with an increase of $AgNO_3$ (see table 84).

If we study the effect of these sols on a protein solution such as that of gelatin we find that the negative silver chloride sols precipitate with positive gelatin, i.e., on the acid side of the isoelectric point of the "free" protein, whereas the positive silver chloride sols precipitate with the gelatin on the alkaline side of its isoelectric point, i.e., with negative gelatin. Since the Cl as well as the H ions decide the sign of charge of the components of these systems, HNO_3, instead HCl, must be used in adjusting the pH.

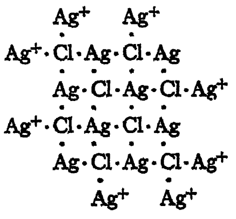
These systems are very complicated, as shown by a single illustration: If a mixture of negative silver chloride and of positive gelatin is negative at pH 4 we know that the silver chloride is in excess and that we can render the system isoelectric by adding more of the gelatin solution. But we can also adopt the activity concept and say that the silver chloride is the most active component for we can also render the system isoelectric either by des-activating the silver chloride with a little Ag NO_3 or by activating the gelatin by a lowering of the pH with HNO_3. If, on the other hand, the mixture is positive at pH 4 it may be made isoelectric: (*a*) by addng more of the silver chloride, (*b*) by activating the silver chloride by a little NaCl, or (*c*) by des-activating the gelatin by raising the pH.

Now what is the nature of this amphoteric compound (or compounds) of silver and chlorine? To express a series of compounds differing so greatly in their behavior by the simple formula AgCl would be absurd. To say that negative silver chloride represents the compound AgCl, made negative and stabilized by NaCl does not account for its behavior. It would be impossible to account for its reaction with positive gelatin chloride on the assumption that mutual flocculation depends merely on the reaction between the "stabilizing" electrolytes. What reaction can take place between NaCl and HCl?

The following hypothesis will be found to account for all the observed phenomena. When crystals of AgCl are forming in a solution both ions must enter the crystal lattice in the same proportion until one of the ions is depleted, then the crystals must cease to grow. But the other ion, if present in excess, will continue to be attracted by the ions of opposite charge in the surface layer of the crystals. Since the ions which are deposited in excess are only partly neutralized the crystal will carry an electric charge and act as a complex anion or cation which may be represented as follows:



Chlor-argentic
anion-complex



Chlor-argentic
cation-complex

The ions in excess which retain a free charge must remain paired, the Cl ions to the Na ions, and the Ag ions to the NO_3 ions. But the Na and NO_3 ions possess a high solution tension and remain dissociated forming the outer layer or ion atmosphere. If, in place of the customary molecular formula, we attempt a formulation of a colloidal ionogen of this type we might represent the ionization by the following scheme:

$$(AgCl)_x \cdot (ClNa)_y \rightleftarrows {}_yNa^+ + (AgCl)_x \cdot Cl_y^-$$

and

$$(AgCl)_x \cdot (AgNO_3)_y \rightleftarrows {}_yNO_3^- + (AgCl)_x \cdot Ag_y^+$$

If, in the preparation of negative silver chloride we use HCl instead of NaCl we would obtain the acid form of the complex $H_y(AgCl)_xCl_y$. This compound is analogous to chlor-auric acid. The complex cyanides of silver are other examples of this type of compounds, the main difference being that these complex ions are soluble.

The values of x and y will depend upon the excess of either reagent. With a large excess of NaCl or $AgNO_3$ the negative or positive charge will be strong and the crystals will remain small. This means a large surface and a relatively large value of y.

When negative silver chloride reacts with positive gelatin chloride a double displacement takes place, NaCl is formed and the chlor-argentic anion unites with the gelatin cation. When positive silver chloride reacts with negative gelatin, i.e., Na-gelatinate, $NaNO_3$ is formed and the chlor-argentic cation unites with the gelatin anion.

THE REACTIONS WITH BARIUM SULFATE

The cataphoresis of barium sulfate has been shown in a previous publication (6). With equivalent quantities of $BaCl_2$ and an alkali sulfate a weakly electropositive precipitate is formed. This is taken to indicate that the Ba ions are more strongly associated in the crystal lattice than the SO_4 ions. An excess of Ba results in a still more positive precipitate, whereas an excess of SO_4 ions yields a negative charge. The positive and negative barium sulfate crystals may be respectively represented as follows:

$(BaSO_4)_x \cdot (Ba)_y^{++}$	$(BaSO_4)_x \cdot (SO_4)_y^{--}$
cation complex	anion complex

The accompanying diffusible anions and cations are displaceable and may consist of any anions or cations originally present in the solution.

Barium sulfate reacts with the proteins in the same manner as silver chloride but the activities of the two salt complexes differ. Silver chloride is more active than barium sulfate and each of the two compounds is more active in the positive than in the negative condition. This is clearly shown in tables 85 and 86.

Table 84 shows that cataphoresis of "free" silver chloride. The isoelectric point could not be determined because in equivalent proportions of $AgNO_3$ and $NaCl$ and in a slight excess of the former the particles migrated in opposite directions. It seemed as if the larger aggregates were predominantly positive

TABLE 84

The $AgNO_3$ + $NaCl$ system (50 cc.)

$AgNO_3$, 0.1 *N*	$NaCl$, 0.1 *N*	FLOCCULATION		μ/SEC. 1 VOLT/CM.	pH
		After mixing	Overnight		
cc.	*cc.*				
2.0	1.5	Opal	+	+2.9	....
2.0	1.9	Opal	++	+2.5	....
2.0	1.95	Opal	+++	Some + Some −	6.3
2.0	2.0	Milky	++++	Some + Some −	6.35
2.0	2.05	Milky	+++	−2.5	6.4
2.0	2.1	Opal	+	−3.0	....
2.0	2.5	Opal	0	.....	....

TABLE 85

<table>
<tr><th rowspan="2">NaOH OR HNO₃</th><th colspan="2">FLOCCULATION</th><th rowspan="2">μ/SEC. 1 VOLT/CM.</th><th rowspan="2">pH</th></tr>
<tr><th>After mixing</th><th>Overnight</th></tr>
<tr><td><i>millimol</i></td><td></td><td></td><td></td><td></td></tr>
<tr><td colspan="5"><i>The (AgCl)_x·(AgNO₃)_y + Na-caseinate system (in 50 cc.)</i>
A: 2 cc. 0.1 <i>N</i> AgNO₃ (+ HNO₃).
B: 1 cc. 0.1 <i>N</i> NaCl + 5 mgm. Na-caseinate (+ NaOH).</td></tr>
<tr><td>0.025 NaOH</td><td>Opal</td><td>+</td><td>−1.32</td><td>8.2</td></tr>
<tr><td>0.015 NaOH</td><td>Slow</td><td>++++</td><td>−0.89</td><td>7.5</td></tr>
<tr><td>0.01 NaOH</td><td>Instant</td><td>++++</td><td>−0.43</td><td>7.2</td></tr>
<tr><td>0.0</td><td>Slow</td><td>++++</td><td>+0.61</td><td>6.5</td></tr>
<tr><td>0.005 HNO₃</td><td>Milky</td><td>+++</td><td>+1.01</td><td>5.8</td></tr>
<tr><td>0.01 HNO₃</td><td>Opal</td><td>0</td><td>......</td><td>4.5</td></tr>
<tr><td colspan="5"><i>The (AgCl)_x·(ClNa)_y + Na-caseinate system (in 50 cc.)</i>
A: 10 cc. 0.1 <i>N</i> AgNO₃.
B: 15 cc. 0.1 <i>N</i> NaCl + 5 mgm. Na-caseinate (NaOH).</td></tr>
<tr><td>0.01 HNO₃</td><td>Opal</td><td>0</td><td>......</td><td>6.0</td></tr>
<tr><td>0.02 HNO₃</td><td>Slow</td><td>++++</td><td>−1.38</td><td>4.4</td></tr>
<tr><td>0.03 HNO₃</td><td>Rapid</td><td>++++</td><td>+ slight</td><td>4.0</td></tr>
<tr><td>0.04 HNO₃</td><td>Rapid</td><td>++++</td><td>+0.38</td><td>3.8</td></tr>
<tr><td>0.05 HNO₃</td><td>Rapid</td><td>++++</td><td>+0.61</td><td>3.6</td></tr>
<tr><td>0.10 HNO₃</td><td>Rapid</td><td>++++</td><td>+0.95</td><td>3.1</td></tr>
</table>

while the smallest were negative. It will be noted that the pH is displaced to the acid side by the positive complex and to the alkaline side by the negative complex. The same observation was made in the barium sulfate system (6). This is explained as follows: In the positive systems the diffusible and displaceable anions are partly displaced by the OH ions thus:

$$(\mathrm{AgCl})_x \cdot (\mathrm{AgNO_3})_y + \mathrm{OH^-} - \mathrm{H^+} \rightleftarrows$$
$$(\mathrm{AgCl})_x \cdot (\mathrm{AgOH})_y + \mathrm{NO_3^-} + \mathrm{H^+}$$

TABLE 86

HCl	FLOCCULATION		μ/SEC. 1 VOLT/CM.	pH
	After mixing	Overnight		
millimol				
The $(BaSO_4)_x \cdot (BaCl_2)_y$ *+ Na-caseinate system (in 50 cc.)* A: 2 cc. 0.1 *N* $\mathrm{BaCl_2}$ (+ HCl). B: 1 cc. 0.1 *N* $\mathrm{Na_2SO_4}$ + 5 mgm. Na-caseinate.				
0.005	None	+	−1.68	6.1
0.01	Slow	+++	−0.82	5.4
0.0125	Slow	+++	+0.67	4.5
0.015	Slow	++	+1.60	4.2
0.02	None	+	+2.08	4.1
0.025	None	+	+2.33	4.0
The $(BaSO_4)_x \cdot (SO_4Na_2)_y$ *+ Na-caseinate system (in 50 cc.)* A: 10 cc. 0.1 *N* $\mathrm{BaCl_2}$ (+ HCl). B: 15 cc. 0.1 *N* $\mathrm{Na_2SO_4}$ + 5 mgm. Na-caseinate.				
0.015	None	0	−2.02	6.8
0.02	Slow	++	−1.89	6.0
0.025	Rapid	++++	±0.0	4.5
0.03	Rapid	++++	+0.87	4.1
0.035	Rapid	++++	+0.87	4.0
0.04	Rapid	++++	+1.04	3.8
0.05	Slow	++++	+1.21	3.4

In the negative systems the diffusible and displaceable cations are partly displaced by the H ions in solution thus:

$$(\mathrm{AgCl})_x \cdot (\mathrm{ClNa})_y + \mathrm{H^+} + \mathrm{OH^-} \rightleftarrows$$
$$(\mathrm{AgCl})_x \cdot (\mathrm{ClH})_y + \mathrm{Na^+} + \mathrm{OH^-}$$

With neutral salts (other than chlorides) the positive complex yields an exchange alkalinity, whereas an exchange acidity is developed by the negative complex. The point of exchange neutrality will, however, vary with the ionic composition of the solution.

As examples of the reactions between silver chloride and barium sulfate on the one side and the proteins on the other, only the caseinated systems will be shown here because the barium sulfate did not flocculate with gelatin as did silver chloride.

It will be noted that the systems in which the silver chloride and barium sulfate act as cationic complexes, i.e., are positive, are taken 10 times as dilute as the systems in which these compounds act an anionic complexes. Systems containing 15 cc. 0.1 *N* $AgNO_3$ to 10 cc. 0.1 *N* NaCl and 15 cc. 0.1 *N* BaCl to 10 cc. 0.1 *N* Na_2SO_4 together with 5 mgm. casein remained positive at all pH values up to 7.2 and 8.6 respectively. The concentrations of the positive silver chloride and barium sulfate were therefore reduced to one-tenth of the concentrations of the negative suspensions, with the result shown in tables 85 and 86. It must be pointed out that the dilution is not the only difference in the two pairs of systems since the excess of NaCl and Na_2SO_4 amounts to 5 cc. 0.1 *N* while that of $AgNO_3$ and $BaCl_2$ is only 1 cc. 0.1 *N*. The negative silver chloride and barium sulfate complexes were therefore not only more concentrated but were also favored by a higher concentration of the activating ions.

The results were a displacement of the isoelectric pH of 4.8 of "free" casein, to about 4.1 by the negative silver chloride, to 4.5 by the negative barium chloride, to about 6.9 by the positive silver chloride, and to about 4.9 by the positive barium chloride. The zones of flocculation were correspondingly displaced.

This all indicates that silver chloride is more active than barium sulfate. We might account for this behavior by saying that the Ag and Cl ions possess a stronger power of association than do the Ba and SO_4 ions. The "excess association" of Ag or Cl ions on the surface of silver chloride crystals will, at a given excess of either ion in solution, be greater than the "excess association" (i.e. adsorption) of Ba or SO_4 ions on the surface of barium sulfate crystals.

As to the greater activity of the positive than of the negative complexes of the two salts, no definite statement can be made, for we might equally well explain the behavior by saying that, over the pH range in question, the negative protein is less active than the positive protein.

THE CHEMISTRY OF PRECIPITATES

The idea that a precipitate represents a definite compound which is chemically inactive and which can react only through its dissolved phase must be rejected. X-ray analysis has shown that the space arrangement within the crystal lattice is that of an equidistant position of single ions; no one pair of ions can be singled out as a molecular species. This being the case it is only rational that the crystal should behave as an ionic species and react with the ions and polar molecules in the external medium by virtue of the free fields of the surface layer of ions. An ion in the solution which forms a strongly associated compound with one of the ions in the crystal will be attracted and "adsorbed" by the crystal. This creates a potential difference which becomes greater the further the association or adsorption of the ion proceeds. The crystal becomes now an active ion complex with a charge of the same sign as that of the ion associated in excess. Thus an excess of Ag or Cl ions will become associated with the silver chloride crystals when either ion is present in excess in the solution. Similarly the Ba or SO_4 ions will associate themselves with the barium sulfate crystals.

The potential of the crystals is thus increased until a critical maximum is attained. The critical potential appears to be at about 70 millivolts or about that calculated by von Hevesy (2) for the common single ions. At this potential any oppositely charged ion in the solution will be attracted to the crystal lattice no matter how high the dissociation constant of the compound in the free state. Thus the chlor-argentic cation complex $(AgCl)_x \cdot Ag_y^+$ will attract the NO_3 ions to form the complex $(AgCl)_x \cdot Ag_y^+ \cdot (NO_3)_{y-z}^-$ with z positive charges, where z represents the number of charges at the critical potential. The ion complex is so highly multivalent that the potential would attain enormous magnitudes if all the diffusible ions dissociated. This is apparently why the saloids (compounds between ionic complexes and single diffusible ions) undergo only a limited dissociation (7) as compared to ordinary salts which can undergo complete dissociation since their ions possess only one or two charges and therefore never exceed the critical potential. (The smaller ions are, as we assume, stabilized by the attraction of water molecules, i.e., hydration.)

When the potential of the ionic complexes approaches a maximum a further increase is retarded not only by the attraction of the otherwise easily dissociated diffusible ions but also by an attraction of the polar water molecules. The hydration, i.e., the electrically attracted water, is greater the higher the potential.

The magnitude of the charge and the hydration accounts for the high degree of dispersion of strongly positive or negative precipitates. The oriented layer of water molecules may be assumed to inhibit the growth of the crystals and the charge may conceivably affect the growth as explained in section III (9). Since the free energy of the system must strive toward a minimum it is obvious that the total surface for the distribution of the charge must tend to remain large whenever one ion is associated in excess of an oppositely charged ion. According to the views presented in section III very small particles having a great curvature will accomodate per unit surface, at a given potential, more ions than larger particles. Crystals or colloidal particles which are strongly positive or negative attain accordingly only submicroscopic dimensions.

The highly charged ion complex, whether crystalline or amorphous particles, will actively combine with ions or other ion complexes of opposite charge with which non-dissociated complex is formed. The original charge may then be completely neutralized or there may be a change in charge depending upon the quantity or activity of the added substance. The reaction must be looked upon as a double displacement, but a stoichiometrical relationship must be accidental and in most cases highly improbable. Cation must, however, displace cation, and anion must displace anion in equivalent quantities in order to maintain electroneutrality of the micelle as a unit.

THE FUNCTION OF THE PROTEINS IN THE SOIL

There can be no doubt that the proteins enter into the make-up of the soil colloidal complex, combining with the basic and acidic constituents according to the pH of the soil solution. But the reactions in the soil are so complex that a discussion of this subject must be postponed until a comprehensive study in this direction has been made of the different horizons of the various soil profiles. Such work is now in progress at the New Jersey Agricultural Experiment Station. The preceding study of simple systems must be looked upon as a necessary forerunner to the study of more complicated systems such as those of the various types of the natural soils.

The behavior of the proteins may account for the high nitrogen content in humus and in the B horizons of soils. Proteins, coagulated in the form of various complexes such as those here studied, may be more resistant to decomposition just as leather, which may be looked upon as "protein tannate," is highly resistant. The use of aluminum salts as "tanning" agents indicates a similar resistance of the aluminum "proteinates." In this connection it is of interest to note that the "silicate of gelatin" prepared by Thomas Graham (1) containing 100 parts of silicic acid to about 92 of gelatin "does not putrify in the humid state."

SUMMARY

This paper deals with the electrokinetic and chemical behavior of aluminum- and ferric-"proteinates" and of protein-"humates," "bentonates," and "silicates." The proteins studied include albumin, casein, edestin, and gelatin.

The complex formation between the proteins on the one side and the positive and negative silver chloride and barium sulfate on the other has also been studied and discussed.

The fundamental principles previously established in the case of the silicated, phosphated, and humated compounds of the sesquioxides were found to apply to all the complexes herein described and have thus been generalized.

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NEWS NOTES

The Chilean Nitrate of Soda Nitrogen Research Award of $1,000 was this year divided equally between Dr. W. H. Pierre of the department of agronomy of the University of West Virginia and Dr. Hans Jenny of the department of soils of the University of Missouri. This award has as its object recognition and encouragement of research on any of the diverse aspects of the rôle of nitrogen in economic crop production. It is administered by a standing committee of the American Society of Agronomy.

The award to Dr. Pierre was made in recognition of his work on the effect of the various nitrogenous fertilizers on soil reaction. His work has clearly shown that the continued use of $(NH_4)_2SO_4$ on light, poorly buffered soils will develop within a few years a degree of acidity which is distinctly harmful to many crops. Similar effects are not obtained with the other common nitrogen fertilizers. By supplementing $(NH_4)_2SO_4$ with sufficient lime, satisfactory results may be obtained.

The award to Dr. Jenny was made in recognition of his work on the effect of climate on the nitrogen content of soils. By a careful correlation of climatic data and of the thousands of nitrogen determinations made on the soils of America, Dr. Jenny has been able to develop an equation of state which shows the general relationship existing between the stable nitrogen content of soils and temperature and rainfall, which are the principal climatic factors affecting it.

In addition to the cash awards, the winners were presented with an appropriate certificate by the American Society of Agronomy.

Members of the International Society of Soil Science are urgently requested to send payments for their 1932 dues at once to Dr. A. G. McCall, Room 125 East Wing, U. S. Department of Agriculture, Washington, D. C. Early in 1932 all mailing lists for publications will be closed and only those members whose payments for dues have reached the office of the general secretary abroad by that time, will be enabled to receive without charge, the Journal and Soil Research as well as volume A of the Proceedings of the Sixth Commission meeting at Groningen, 1932. You cannot afford to miss the opportunity to obtain the Transactions of the Second Congress held in Russia, at the special price accorded to members. So please send your remittance promptly.

ELECTROKINETICS AND BASE-EXCHANGE CAPACITY OF SOME INORGANIC COLLOIDS

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Working with series of isoelectric precipitates of phosphates, silicates, and humates of iron and aluminum, Mattson (4) has embodied the behavior of these substances in the generalized statement, "The adsorption and exchange of cations, at pH 7.0, by soil colloids, in the same state of aggregation, increases with the increasing ratio of the acidoid to the ampholytoid constituents of the colloid." The members of these series of colloids, in which the ratio of humate, phosphate, or silicate, to iron and aluminum is greatest, are isoelectric at lowest pH, and have the largest exchange capacity. The seat of this capacity for adsorption and exchange of cations, at favorable pH ranges, lies in the free valences of the partially linked-up, yet stably combined anions.

In this work a series of isoelectric precipitates of "ferric ferrocyanide" and "sulfide of tin", and electronegative "sulfides" of antimony, bismuth, and arsenic, were prepared and their base-exchange capacities investigated.

In finding the isoelectric point of the various flocs, definite volumes of solutions A and B were measured, one into a large pyrex test tube, the other into a beaker. When required, HCl or NaOH was respectively added to the acid A, or alkaline B solutions, to give a range of pH values in the mixtures. Varying amounts of water were also added, so that the final volume of the mixtures of the same series was identical. The mixing was done by rapidly pouring one solution into the other, then back and forth between the vessels, and finally into the test tube, which was then stoppered. The flocculation immediately after the solutions were mixed, and after standing overnight, is recorded in the tables. The latter data is represented by crosses; x signifies slight, xx more complete, xxx almost complete, and xxxx complete flocculation. The pH was determined colorimetrically.

After standing overnight the tubes were shaken and cataphoretic measurements were made as described elsewhere (1).

A series of ferric ferrocyanide was prepared in which the equivalent ratios of the ferric and ferrocyanide constituents were: 15:2, 15:5, 15:10, 15:15, 15:20. By varying the pH in these series, an isoelectric compound in all but the latter ratio was found. Details of this procedure together with flocculation effects, cataphoretic measurements, and pH measurements are given in systems 1–5.

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[1] This work was performed during a short visit by N. H. Parbery to New Jersey Agricultural Experiment Station. Journal Series paper of the New Jersey Agricultural Experiment Station, Department of Soil Chemistry and Bacteriology.

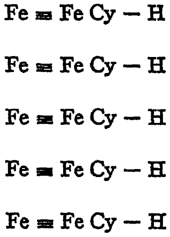
It has been shown (2) that the isoelectric point of ferric hydroxide is at pH 7.1 —at or below this pH, the capacity of this compound for absorbing bases is negligible.

The introduction of a small amount of ferrocyanide ion into ferric hydroxide by displacement of some of the hydroxyl ion, has a marked effect in displacing the isoelectric pH of the new compound, from that of ferric hydroxide. In system 1 it will be seen that the isoelectric point is at pH 4.7. In system 2, the compound produced by the displacement of more hydroxyl ion by ferrocyanide is isoelectric at pH 4.3, and in system 3 at pH 3.9. Ferric ferrocyanide behaves then, in similar manner to the previously mentioned colloids in that, with increasing ratio of ferrocyanide to iron, the pH at the isoelectric point becomes progressively lower. The series in which stannous hydroxide is increasingly saturated with sulfide sulfur, exhibits this feature in a still more decided fashion. Both the isoelectric ferrocyanides of iron and the isoelectric sulfides of tin form natural series in which the various factors and functions vary in an ascending or descending order.

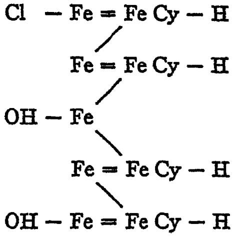
System 4 containing equivalent proportions of each constituent is isoelectric at pH 4.4 which is higher than that of system 3. The cause of this reversal, if real, has not been determined. It will be noted that instant flocculation begins in system 4 at a lower pH than in systems $3<2<1$. The descending order in respect to flocculation is therefore intact.

In system 5 no isoelectric point was found. Even at very low pH there was no flocculation, and feldspar particles suspended in the solutions were negatively charged. Although the ferrocyanide ion was stoichiometrically in excess of the ferric ion no ferrocyanide remained in solution when the flocculation was forced by the addition of $CaCl_2$. The flocculated electronegative complex contained no Cl ions whereas all the isoelectrically flocculated ferric ferrocyanide complexes contained appreciable quantities of Cl. The electronegative complex in system 5 might therefore be represented by formula (*A*). The Cl ions are here all displaced by the ferrocyanide and at low pH the OH ions, being greatly suppressed in the solution, would offer no competition and be practically absent in the complex. This complex must therefore remain electronegative in the first place, because of the absence of dissociable anions (e.g., Cl ions) and in the second place because of the presence of a great number of dissociable cations which exist in combination with the "free" valences of the ferrocyanide.

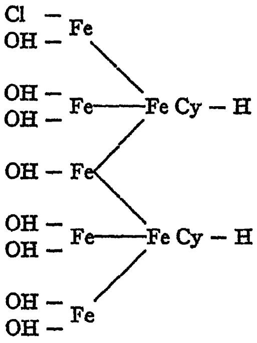
The other ferric-ferrocyanide complexes which all exhibit an amphoteric behavior, i.e., possess an isoelectric point, may all be represented in a general way by formulas (*B*, *C*, and *D*). These complexes contain OH and Cl as well as ferrocyanide anions. To account for the strong negative charge at the higher pH and for the cation exchange capacity (table 1 b) we must assume the existence of "free" ferrocyanide valences which exist in combination with dissociable and displaceable cations. These complexes represent therefore partly hydrolyzed salts. Since the products of the partial hydrolysis are nondiffusible and remain in the complex, the latter contains both an acid and a basic residue. In other words the complex constitutes an amphoteric colloid (5).



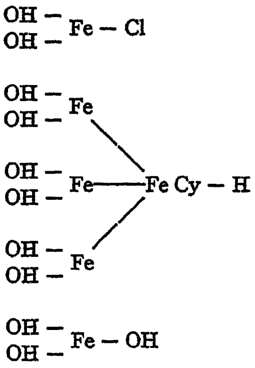
(*A*) negative complex



(*B*) amphoteric complex low isoelectric pH



(*C*) amphoteric complex intermediate isoelectric pH



(*D*) amphoteric complex high isoelectric pH

Whenever the dissociable anion (i.e., Cl) is displaced by a non-dissociable or non-diffusible anion the isoelectric pH must be displaced to the acid side: (*a*) because the dissociation of the basic group of the complex is thereby suppressed until, at a lower pH, a sufficient additional number of the OH ions of the basoid are displaced by dissociable anions, and (*b*) if the displacing anion is di- or polyvalent it will, if it forms a weak acid, only partly link itself to the basoid group, one or more of its valences remaining in combination with hydrogen or other dissociable cations. The result is a new amphoteric complex possessing: a stronger acid group, a weaker basic group, a lower isoelectric pH, a higher cation exchange capacity at a given pH on the alkaline side, and a lower anion exchange capacity at a given pH on the acid side of the isoelectric point.

STANNOUS SULFIDE

In system 6 it will be seen that the isoelectric point of stannous hydroxide, when formed from stannous chloride, is at pH 6.6.

A series of isoelectric sulfides was prepared in which there was a progressive saturation of the oxychloride of tin with sulfide sulfur. As saturation with sulfide sulfur becomes more complete, the isoelectric points of the members of the series are at increasingly low pH values. Unlike the ferrocyanide complexes, which possess a stronger acid residue and are isoelectric at low pH, sufficient dissociable Cl enters the stannous oxysulfide complex, when one-fifth saturated with sulfide sulfur, and renders it isoelectric at pH 6.2. When fully "saturated" with sulfide, stannous sulfide is isoelectric at pH 3.2.

TABLE 1

$FeCl_3 + Na_4[Fe(CN)_6]$—*system 1*

A. 7.5 m.e. $FeCl_3$ per liter.
B. 1.0 m.e. $Na_4[Fe(CN)_6]$ per liter.

SOLUTION A	SOLUTION B, 20 cc. + NaOH (1 cc. = 0.02 M.E. NaOH)*	FLOCCULATION		μ/SEC. 1 VOLT/CM.	pH
		On mixing	Overnight		
cc.	*cc.*				
20	1.5	Deep blue, none	None	......	.....
20	2.0	Blue, none	None	......	.....
20	3.0	Purplish blue, none	None	......	.....
20	4.0	Purple, none	xxx	+2.16	<3.0
20	5.0	Mauve, none	xxxx	+1.51	<3.0
20	5.25	Brown-mauve, none	xxxx	+1.44	3.4
20	5.50	Brown-mauve, none	xxxx	+1.26	3.8
20	5.75	Mauve-brown, slow	xxxx	+1.21	4.1
20	5.88	Mauve-brown, rapid	xxxx	+0.87	4.4
20	6.0	Mauve-brown, instant	xxxx	+0.67	4.5
20	6.13	Mauve-brown, instant	xxxx	+0.10	4.6
20	6.18	Mauve-brown, instant	xxxx	±0.0	4.7
20	6.25	Mauve-brown, instant	xxxx	−0.15	4.8
20	6.50	Brown, instant	xxxx	−1.51	6.0
20	7.0	Brown, none	xxxx	−2.52	7.0

* Plus water to give the mixture a total volume of 50 cc.

Isoelectric mixture: 7.5 m.e. $FeCl_3$ per liter.
1.0 m.e. $Na_4[Fe(CN)_6]$ + 6.18 m.e. NaOH in 1,500 cc.
pH 4.7.
Fe and $Fe(CN)_6$ in solution, nil.

Composition of floc: $Fe_4(OH)_{10.268}[Fe(CN)_6]_{0.4}Cl_{0.132}$.

In these formulas no allowance is made for the acid and basic residues resulting from hydrolysis as indicated in formulas (*B*, *C*, and *D*).

When sodium sulfide was added in excess of equivalent proportions to stannous chloride, flocculation did not result and acidification was necessary to bring it about. No isoelectric point of this compound was found. This is because in acid solution, and in the presence of excess sulfide ions, the anion adsorption capacity of the floc is satisfied by sulfide ions, to the exclusion of dissociable chloride ions. Thus an isoelectric or electropositive compound,

TABLE 2

$FeCl_3 + Na_4[Fe(CN)_6]$—system 2

A. 7.5 m.e. $FeCl_3$ per liter.
B. 2.5 m.e. $Na_4[Fe(CN)_6]$ per liter.

SOLUTION A	SOLUTION B, 20 cc. + NaOH (1 cc. = 0.02 M.E. NaOH)	FLOCCULATION		μ/SEC. 1 VOLT/CM.	pH
		On mixing	Overnight		
cc.	*cc.*				
20	2.5	Blue, none	xxxx	+1.59	<3.0
20	3.0	Blue, none	xxxx	+1.47	3.0
20	3.5	Blue, none	xxxx	+1.21	3.2
20	4.0	Blue, slow	xxxx	+0.86	3.6
20	4.5	Blue, instant	xxxx	+0.20	4.1
20	4.6	Blue, instant	xxxx	±0.0	4.3
20	5.0	Greenish blue, instant	xxxx	−1.26	4.8
20	5.5	Bluish green, none	None	......	...
20	6.0	Blue-gray, none	None	......	...
20	6.5	Gray-mauve, none	None	......	...
20	7.0	Gray-brown, none	xx	......	...

Isoelectric mixture: 7.5 m.e. $FeCl_3$ per liter.
2.5 m.e. $Na_4[Fe(CN)_6]$ + 4.6 m.e. NaOH in 1,500 cc.
pH 4.3.
Fe and $Fe(CN)_6$ in solution, nil.
Composition of floc: $Fe_4(OH)_{7.84}[Fe(CN)_6]_{1.0}\ Cl_{0.16}$.

TABLE 3

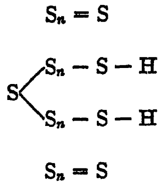
$FeCl_3 + Na_4[Fe(CN)_6]$—system 3

A. 7.5 m.e. $FeCl_3$ per liter.
B. 5.0 m.e. $Na_4[Fe(CN)_6]$ per liter.

SOLUTION A	SOLUTION B, 20 cc. + NaOH (1 cc. = 0.01 M.E. NaOH)	FLOCCULATION		μ/SEC. 1 VOLT/CM.	pH
		On mixing	Overnight		
cc.	*cc.*				
20	6.0	Blue, none	None	......	...
20	5.0	Blue, very slow	xxxx	−1.51	5.0
20	4.8	Blue, instant	xxxx	−1.21	4.6
20	4.6	Blue, instant	xxxx	−0.89	4.5
20	4.4	Blue, instant	xxxx	−0.61	4.3
20	4.2	Blue, instant	xxxx	−0.41	4.2
20	4.0	Blue, instant	xxxx	−0.22	4.1
20	3.8	Blue, instant	xxxx	− slight	4.0
20	3.6	Blue, instant	xxxx	±0.0	3.9
20	3.4	Blue, instant	xxxx	......	3.8
20	3.2	Blue, instant	xxxx	+0.19	3.7
20	3.0	Blue, instant	xxxx	+0.30	3.6

Isoelectric mixture: 7.5 m.e. $FeCl_3$ per liter.
5.0 m.e. $Na_4[Fe(CN)_6]$ + 1.8 m.e. NaOH in 1,500 cc.
pH 3.9.
Fe and $Fe(CN)_6$ in solution, nil.
Composition of floc: $Fe_4(OH)_{3.6}[Fe(CN)_6]_{2.0}\ Cl_{0.40}$.

except perhaps in very strongly acid solution, could not be obtained. The following formula illustrates the probable structure of this sulfide.



In preparing the series of sulfides, the amount of stannous chloride was kept constant, and varying amounts of sodium sulfide (plus varying amounts of HCl or NaOH to give pH ranges for each compound) were added, so as to give ratios of tin and sulfide, expressed on a milliequivalent basis of 5:1, 5:2, 5:3, 5:4, 5:5, 5:6.

TABLE 4

$FeCl_3 + Na_4[Fe(CN)_6]$—system 4

A. 7.5 m.e. $FeCl_3$ per liter.
B. 7.5 m.e. $Na_4[Fe(CN)_6]$ per liter.

SOLUTION A	SOLUTION B, 20 cc. + NaOH (1 cc. = 0.02 M.E. NaOH)	FLOCCULATION On mixing	FLOCCULATION Overnight	μ/SEC. 1 VOLT/CM.	pH
cc.	*cc.*				
20	7	Light brown, none	None	......	...
20	5	Green, none	None	......	...
20	1	Greenish blue, none	None	......	...
SOLUTION A, 20 cc. + HCl (1 cc. = 0.01 M.E. HCl)	SOLUTION B				
cc.	*cc.*				
0.2	20	Blue, none	xxxx	−1.51	5.2
0.4	20	Blue, very slow	xxxx	−1.21	4.6
0.6	20	Blue, very slow	xxxx	±0.0	4.4
0.8	20	Blue, very slow	xxxx	+0.15	4.2
1.0	20	Blue, slow	xxxx	+0.17	4.1
1.4	20	Blue, instant	xxxx	......	3.9
1.6	20	Blue, instant	xxxx	......	3.8
1.8	20	Blue, rapid	xxxx	+0.17	...
5.0	20	Blue, slow	xxxx	+0.30	<3.0

Isoelectric mixture: $\begin{cases} 7.5 \text{ m.e. } FeCl_3 \text{ per liter.} \\ 7.5 \text{ m.e. } Na_4[Fe(CN)_6] + 0.3 \text{ m.e. HCl in 1,500 cc.} \end{cases}$
pH 4.4.
Fe and $Fe(CN)_6$ in solution, nil.
Composition of floc: $Fe_4[Fe(CN)_6]_3Cl_{0.418}$.

As already stated, the last-mentioned ratio was the only one in which an isoelectric point was not found.

TABLE 5

$FeCl_3 + Na_4[Fe(CN)_6]$—*system 5*

A. 7.5 m.e. $FeCl_3$ per liter.
B. 10.0 m.e. $Na_4[Fe(CN)_6]$ per liter.

SOLUTION A, 20 cc. + HCl (1 cc. = 0.01 M.E. HCl)	SOLUTION B	FLOCCULATION		μ/SEC. 1 VOLT/CM.	pH
		On mixing	Overnight		
cc.	*cc.*				
2	20	Blue, none	None	.....	.....
4	20	Blue, none	None	.....	.....
6	20	Blue, none	None	.....	.....
8	20	Deeper blue, none	None*	.....	.....
10	20	Deeper blue, none	None†	.....	.....
SOLUTION A, 20 cc. + HCl (1 cc. = 0.10 M.E. HCl)					
cc.					
2	20	Deeper blue, none	None	.....	.....
3	20	Deeper blue, none	None	.....	.....
10	20	Deepest blue, none	None	.....	<3.0

* When flocculated with $CaCl_2$, floc negatively charged.

† Feldspar particles suspended in solution were negatively charged.

There is neither isoelectric point nor flocculation even at very low pH.

Complete flocculation of mixture at low pH was effected with $CaCl_2$, and no Fe or $Fe(CN)_6$ remained in solution.

TABLE 6

$SnCl_2 + NaOH$—*system 6*

A. 8.0 m.e. $SnCl_2$ per liter.

SOLUTION A	NaOH IN 25 cc. (1 cc. NaOH = 0.025 M.E.)	FLOCCULATION		μ/SEC. 1 VOLT/CM.	pH
		On mixing	Overnight		
cc.	*cc.*				
25	9.0	Slight opalescence, none	None	......	<4.0
25	10.05	Opalescence, none	x	+1.51	5.5
25	10.2	Slow	xxxx	+1.73	6.3
25	10.3	Instant	xxxx	+1.44	6.4
25	10.4	Instant	xxxx	+1.01	6.5
25	10.6	Opalescence, none	x	−0.92	6.7
25	11.0	Slight opalescence, none	x	−0.93	6.9
25	14.0	None	None	......	>8.0

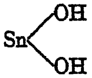
Isoelectric mixture: $\begin{cases} \text{8 m.e. } SnCl_2 \text{ per liter.} \\ \text{10.5 m.e. NaOH per liter.} \end{cases}$
pH 6.6.

The amount of chlorine adsorbed by the compounds at their isoelectric points was not determined, but from analogy with the behavior of ferric ferrocyanide, the lower the pH at the isoelectric point of the compound the greater will be the amount of chlorine adsorbed.

The following formulae illustrate chlorine adsorption.

The first represents stannous hydroxide, isoelectric at high pH, and therefore adsorbing a small amount of chlorine, and the second, stannous hydroxide partly saturated with sulfide ion, isoelectric at lower pH, and adsorbing more chlorine.

$Cl-Sn-OH$ (*E*)



$OH-Sn-S-H$ (*F*)

$Cl-Sn-OH$

$Cl-Sn-S-H$

Stannous hydroxide, or stannous oxychloride, and the various sulfides as prepared, flocculated only over a comparatively narrow pH range. These compounds are dispersed on the acid and alkaline side of the isoelectric point. At the isoelectric point some of the higher ratio sulfides behave in unstable fashion, and the passage of electric current for a very short period will cause the particles to assume various charges. It is conceivable that a colloidal particle, partially saturated with sodium at the moment the current passes through the cataphoresis tube, undergoes electrolysis and becomes hydrogen-saturated, with an accompanying change of pH and charge.

ARSENIOUS SULFIDE

Arsenious sulfide has long been quoted as a classical example of a negative colloid.

Following the methods for the preparation of ferrocyanide series, an attempt was made to prepare a natural series in which there would be an increasing saturation of arsenious oxide with sulfide sulfur.

Definite quantities of a solution of sodium sulfide (standardized with respect to sulfide sulfur) containing 200 m.e. sulfide sulfur per liter, and a solution of arsenious oxide containing 400 m.e. As_2O_3 and 400 m.e. HCl per liter, were mixed and made up to 1 liter. The ratios of sulfur to As_2O_3 on a milliequivalent basis were: 30:25, 25:25, 20:25, 15:25, 25:25, 10:50.

In the first two mixtures flocculation was incomplete and acidification was necessary for complete precipitation. A solution of a sodium polysulfide, the composition of which approximated Na_2S_3 was standardized with respect to sulfide sulfur, and mixtures were made in liter volumes in which the ratios were 30 m.e. sulfide sulfur: 25 m.e. As_2O_3, 30:30, and 10:30. Again in the two highest ratios acidification was necessary for complete precipitation.

In all mixtures except the two cases where sulfide was in excess of arsenic, the precipitation of sulfide was complete—it combined with nearly an equivalent

TABLE 7

$SnCl_2 + Na_2S$—system 7

A. 8 m.e. $SnCl_2$ per liter.
B. 1.6 m.e. Na_2S per liter.

SOLUTION A	SOLUTION B, 25 CC. + NaOH (1 CC. = 0.025 M.E. NaOH)*	FLOCCULATION		μ/CM. 1 VOLT/CM.	pH
		On mixing	Overnight		
cc.	*cc.*				
25	7.0	None	None	......	...
25	8.6	Slow	xxxx	+0.61	5.8
25	8.8	Instant	xxxx	±0.0	6.2
25	9.0	Instant	xxxx	−0.15	7.4
25	9.4	Slow	xxxx	−0.24	6.7
25	9.5	Very slow	Opalescent	−1.04	6.8
25	9.6	None	None	......	6.9

* Plus water to give the mixture a total volume of 60 cc.

Isoelectric mixture: 8 m.e. $SnCl_2$ per liter.
1.6 m.e. Na_2S + 8.8 m.e. NaOH in 1,400 cc.
pH 6.2.
Tin and sulfide in solution, nil.

Composition of floc: $Sn(OH)_{1.6-x}\ S_{0.2}\ Cl_x$.

In these formulas no allowance is made for the free acid residue which undoubtedly exists, as indicated by formula (*F*).

TABLE 8

$SnCl_2 + Na_2S$—system 8

A. 8 m.e. $SnCl_2$ per liter.
B. 3.2 m.e. Na_2S per liter.

SOLUTION A	SOLUTION B, 25 CC. + NaOH (1 CC. = 0.025 M.E. NaOH)	FLOCCULATION		μ/SEC. 1 VOLT/CM.	pH
		On mixing	Overnight		
cc.	*cc.*				
25	5.0	None	None	......	...
25	7.2	Instant	xxxx	+0.61	5.8
25	7.3	Instant	xxxx	+0.38	6.1
25	7.35	Instant	xxxx	±0.0	6.2
25	7.4	Instant	xxxx	−0.76	6.2
25	7.5	Instant	xxxx	......	6.4
25	8.0	None	None	......	...

Isoelectric mixture: 8 m.e. $SnCl_2$ per liter.
3.2 m.e. Na_2S + 7.35 m.e. NaOH in 1,400 cc.
pH 6.2.
Tin and sulfide in solution, nil.

Composition of floc: $Sn(OH)_{1.2-x}\ S_{0.4}\ Cl_x$.

amount of As_2O_3 and an approximate stoichiometric As_2S_3 was formed. Where arsenic was in excess, nearly the estimated excess remained in solution.

TABLE 9

$SnCl_2$ + Na_2S—system 9

A. 8.0 m.e. $SnCl_2$ per liter.
B. 4.8 m.e. Na_2S per liter.

SOLUTION A	SOLUTION B, 25 cc. + NaOH (1 cc. = 0.025 M.E. NaOH)	FLOCCULATION		μ/SEC. 1 VOLT/CM.	pH
		On mixing	Overnight		
cc.	*cc.*				
25	3.0	None	None	......	...
25	5.3	Instant	xxxx	+0.43	4.6
25	5.4	Instant	xxxx	±0.0	5.2
25	5.5	Instant	xxxx	−0.24	5.4
25	5.6	Instant	xxxx	−0.40	5.6
25	6.5	None	None	......	...

Isoelectric mixture: {8.0 m.e. $SnCl_2$ per liter.
{4.8 m.e. Na_2S + 5.4 m.e. NaOH in 1,400 cc.
pH 5.2.
Tin and sulfide in solution, nil.
Composition of floc: $Sn(OH)_{0.8-x}S_{0.6}\,Cl_x$.

TABLE 10

$SnCl_2$ + Na_2S—system 10

A. 8.0 m.e. $SnCl_2$ per liter.
B. 6.4 m.e. Na_2S per liter.

SOLUTION A	SOLUTION B, 25 cc. + NaOH (1 cc. = 0.025 M.E. NaOH)	FLOCCULATION		μ/SEC. 1 VOLT/CM.	pH
		On mixing	Overnight		
cc.	*cc.*				
25	2.0	Instant	xxxx	+0.76	...
25	3.65	Instant	xxxx	+0.38	4.0
25	3.70	Instant	xxxx	+0.30	4.1
25	3.75	Instant	xxxx	±0.0*	4.3
25	3.8	Instant	xxxx	−slight	4.4
25	4.0	Instant	xxxx	−0.60	4.6

* Particles behave indefinitely, becoming variously charged as a result of electrolysis.

Isoelectric mixture: {8.0 m.e. $SnCl_2$ per liter.
{6.4 m.e. Na_2S + 3.75 m.e. NaOH in 1,400 cc.
pH 4.3.
Tin and sulfide in solution, nil.
Composition of floc: $Sn(OH)_{0.4-x}S_{0.8}\,Cl_x$.

The composition of sulfides of arsenic approaches more closely a stoichiometric relationship in the less acid mixtures. The fact that in the most acid solutions the composition departs farthest from theoretical proportions, can be explained by the fact that, apart from solubility effects, there would be a greater tendency in the more acid solutions for adsorption by the compounds of sulfide anion. Thus arsenious sulfide itself adsorbs sulfide ions in acid solution and so an excess of sulfide appears to combine with its equivalent of As_2O_3. Cataphoretic studies were made of all the sulfides, and without exception, even at low pH, they were strongly electronegative. The excess of HCl in the more acid mixtures interfered with cataphoretic determinations, and dilutions had to be made.

TABLE 11

$SnCl_2 + Na_2S$—system 11

A. 8 m.e. $SnCl_2$ per liter.
B. 8 m.e. Na_2S per liter.

SOLUTION A	SOLUTION B, 25 cc. NaOH (1 cc. = 0.025 M.E. NaOH)	FLOCCULATION		μ/SEC. 1 VOLT/CM.	pH
		On mixing	Overnight		
cc.	cc.				
25	0.4	Instant	xxxx	+0.20	3.0
25	0.5	Instant	xxxx	±0.0	3.2
25	0.6	Instant	xxxx	−0.28	...
25	0.7	Instant	xxxx	−0.40	...
25	3.0	None	None	......	4.4

Isoelectric mixture: 8 m.e. $SnCl_2$ per liter.
8 m.e. Na_2S + 0.5 m.e. NaOH in 1,400 cc.

TABLE 12

The composition of arsenious sulfide formed under various conditions

RATIO OF S TO As_2O_3	FROM SODIUM SULFIDE	FROM SODIUM POLYSULFIDE
m.e.		
25:25	$As_{1.892}S_3$	
20:25	$As_{1.901}S_3$	
15:25	$As_{1.880}S_3$	
10:25	$As_{1.898}S_3$	
10:50	$As_{1.830}S_3$	
30:30		$As_{1.980}S_3$
10:30		$As_{1.854}S_3$

Suspensions of arsenious oxide in water are electronegative. In dilute HCl it is also electronegative, though it was thought that the momentary attachment of chloride ions to the As_2O_3 particle during its solution, and the consequent dissociation of chloride ions ($As_2^+O_2Cl + Cl^-$) might render the compound positive. It appears, however, that the attachment of the chloride ion results in an immediately soluble product. The oxide in water is acidic and dissociates diffusible cations, and so renders the oxide electronegative.

$$H_2 As O_3 + H^+$$

Arsenic appears to be too acidic (like phosphorus) to exist as a colloidal cation such as iron, tin, antimony, and other distinctly metallic elements.

ANTIMONY SULFIDE

In the case of the metals iron and tin, the oxychlorides are isoelectric at pH 7.1 and 6.6 respectively. The oxychloride of antimony is isoelectric at pH 5.6, and differs from that of iron and tin in that it contains much more chlorine at the isoelectric point, and its chlorine is in a much more rigidly associated condition. It will be seen that despite the high chlorine content of the oxychloride of antimony, a comparatively low pH is necessary for the complex to yield dis-

TABLE 13

$SbCl_3 + NaOH$—system 12

A. 2,000 m.e. $SbCl_3$ per liter.

SOLUTION A (1 CC. = 2.0 M.E. $SbCl_3$)	NaOH (1 cc. = 0.2 M E. NaOH) MADE UP TO 599 CC.	FLOCCULATION		μ/SEC 1 VOLT/CM.	pH
		On mixing	Overnight		
cc.	*cc.*				
1	25.90	Instant	xxxx	+1.01	4.3
1	25.95	Instant	xxxx	+0.82	4.8
1	26.00	Instant	xxxx	+0.64	5.4
1	26.05	Instant	xxxx	±0.0	5.6
1	26.30	Instant	xxxx	−1.01	5.8
1	26.80	Instant	xxxx	−1.01	6.4
1	26.90	Instant	xxxx	−0.92	6.8
1	27.00	Instant	xxxx	−0.76	7.0
1	27.30	Instant	xxxx	−1.01	7.2

Isoelectric mixture: 2 m.e. $SbCl_3$ + 5.21 m.e. NaOH in 600 cc. pH 5.6.

TABLE 14

$SbCl_3 + Na_2S$—system 13

A. 2,000 m.e. $SbCl_3$ per liter.
B. 1.6 m.e. Na_2S per liter.

SOLUTION A (1 CC. = 2.0 M.E. $SbCl_3$)	NaOH (1 cc. = 0.2 M.E NaOH) ADDED TO 250 CC. SOLUTION B, AND DILUTED TO 599 CC.	FLOCCULATION		μ/SEC. 1 VOLT/CM.	pH
		On mixing	Overnight		
cc.	*cc.*				
1	20.0	Instant	xxxx	−2.33	<3.0
1	21.2	Instant	xxxx	......	<3.0
1	22.8	Instant	xxxx	−2.53	3.4
1	23.0	Instant	xxxx	−2.43	3.6
1	23.2	Instant	xxxx	−1.38	3.8
1	23.4	Instant	xxxx	−2.02	4.0

No isoelectric point was found even at low pH.

sociable chloride ions. Difficulty was experienced with solutions of S_bCl_3, which can only be kept molecularly dispersed in strong HCl solutions. An aqueous solution containing 8 m.e. S_bCl_3 per liter could not be made up and it was necessary to make up a solution containing 2,000 m.e. S_bCl_3 per liter, take 1 cc. of this solution on a watch glass and plunge it into a large volume of water + NaOH, or NaOH + Na_2S, in the preparation of the series, to prevent its premature hydrolysis.

A natural series of isoelectric sulfides of antimony, as prepared in the case of tin, cannot be prepared.

Compounds were prepared in which the ratios of antimony to sulfide sulfur on a milliequivalent basis were: 5:1, 5:2, 5:3. All these compounds were electronegative in the pH ranges examined. They could not be examined in very acid solutions because the evolution of gas at the electrodes prevented cataphoretic measurements.

TABLE 15

$BiCl_3$ + NaOH—system 14

A. 2,000 m.e. $BiCl_3$ per liter.

SOLUTION A (1 CC. = 2.0 M.E. $SbCl_3$)	NaOH (1 CC. = 0.2 M.E. NaOH) IN 599 CC. SOLUTION	FLOCCULATION		μ/SEC. 1 VOLT/CM.	pH
		On mixing	Overnight		
cc.	*cc.*				
1	8	Instant	xxxx	−slight	2.8
1	12	Instant	xxxx	−slight	3.2
1	14	Instant	xxxx	−0.76	3.6
1	15	Instant	xxxx	−slight	4.5
1	16	Instant	xxxx	−1.38	5.8

No isoelectric point was found even at low pH.

It was not ascertained whether the sulfide ion displaced the oxygen or chlorine of the oxychloride, but the entry of a small amount of sulfide, as in system 13, suffices to inhibit the compound from dissociating anions, even at low pH ranges.

BISMUTH OXYCHLORIDE

In bismuth oxychloride, the extreme condition is met among the metals under review, in that there is no isoelectric point for this compound in as acid solutions as examination was possible. In preparing the oxychloride, similar methods to that adopted in the preparation of antimony oxychloride were adopted, because of the readiness with which B_iCl_3 hydrolyzes. The chloride portion of the compound is extremely rigidly held, as no anions appear to be dissociated in acid solution, and the oxychloride is strictly electronegatively charged.

Sulfides of bismuth were prepared and their base exchange properties determined, but they were not examined cataphoretically. It can fairly be assumed, however, from the behavior of the sulfides of antimony that bismuth oxychloride saturated in any degree by sulfide ion, would be electronegatively charged.

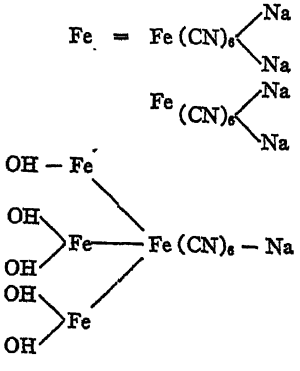
BASE EXCHANGE CAPACITY

After the isoelectric point of the various compounds had been determined, a large volume of each of the reagents was mixed in exactly the same proportions obtaining at the isoelectric point. These mixtures are given with each system where an isoelectric point was determined.

The mixtures in which ferrocyanide was in excess of iron (system 5); sulfide in excess of tin; and the sulfides of arsenic, antimony, and bismuth were all prepared in solutions made acid with HCl, for the isoelectric point of all these compounds, if existent, would be found in acid solution. The base exchange capacity of a substance is here regarded as the amount of exchangeable cation which a substance is capable of adsorbing at pH 7.

Cationic adsorption is brought about by a combination of bases with the acidic constituent of colloids. Cationic adsorption is most in evidence at high pH values—such are necessary to produce hydrolysis, by which process OH ions displace some of the valences of the acidoid component. The acidoid component is not itself displaced, however, but the number of its linkages with the basic constituent is reduced, and it must satisfy its valence requirements by linking up with cations in solution. The seat of cation adsorption and exchange reactions lies in the free valences of these partially linked-up, yet stably combined anions. It will be seen that in the ferric ferrocyanides, in which the ratio of acidoid to basoid is highest, the adsorption of cations is greatest. In these compounds there is the greatest degree of hydrolysis, which means that a larger molar fraction of the acidoid constituent is free to enter into exchange reactions.

The following formula, although not meant to represent what happens in any one molecule of ferric ferrocyanide, illustrates a high degree of hydrolysis of that compound with an accompanying high potential capacity for cation adsorption, represented by sodium ions.



(G)

The precipitates were filtered, dried at 60°C., and where sufficient material was available, about 1 gm. of the finely divided substance was used. This material was treated overnight with 50 cc. neutral *N*Ba—acetate in stoppered flasks, and then leached on the filter with *N*Ba—acetate solution adjusted to pH 7, until the filtrate had a neutral reaction. Leaching was continued somewhat further, 10 cc. *N* $BaCl_2$ adjusted to pH 7 was added, and the material washed entirely free of chloride ions with cooled boiled water. The adsorbed barium was then displaced with *N* NH_4Cl solution at 70°C. and determined as $BaSO_4$.

The base exchange capacity of the ferrocyanides was first determined by treating them by similar methods to the above, but with neutral *N* Ca-acetate. At this pH very considerable hydrolysis of the higher ratio compounds took place, and the competition of OH and acetate ions resulted in the removal of much $Fe(CN)_6$ ion, and the formation of a more basic compound, with a considerably lower exchange capacity. In all but the lowest ratio compound, which was comparatively stable, it was found difficult to bring the filtrate back to pH 7. The extent of the hydrolysis is reflected in the base exchange figures—all the compounds have a similar capacity for exchange, since the acidoid portion of the higher ratio compounds, which is responsible for the exchange phenomenon, has been largely lost. In washing free of Ca-acetate, there was a considerable hydrolysis of the calcium adsorbed by the higher ratio compounds. When washing was complete calcium was still being lost from these compounds but the most basic compounds were not losing calcium by hydrolysis.

TABLE 16

Cation exchange capacities of ferric ferrocyanides at various pH values, expressed in milliequivalents per gram of material

RATIO OF IRON TO FERROCYANIDE	NORMAL SALT TREATMENT		
	Ca-acetate, pH 7	Ba-acetate, pH 6.2	Ba-acetate, pH 5.5
m.e.			
15:2	0.380	0.428	0.046
15:5	0.334	0.563	0.051
15:10	0.426	0.678	0.092
15:15	0.421	0.971	0.384
15:20	0.450	0.677	0.557

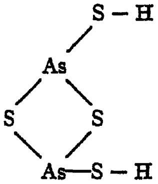
Fresh samples of these compounds were treated with *N* Ba-acetate, since barium has a higher adsorption energy than calcium. In one experiment *N* Ba-acetate was adjusted to pH 6.2 with acetic acid, and in another to pH 5.5. At pH 6.2 all compounds but the highest ratio one were stable, and this compound proved stable at pH 5.5. With the exception of this compound, the base exchange capacities of the ferrocyanides of iron at pH 6.2 increase with the increasing ratio of the acidoid to the ampholytoid component. At pH 5.5, where all compounds are stable, this feature, which is common to many colloids studied, is more strikingly illustrated. The base exchange capacity varies from 0.557 m.e. per gram in the highest ratio compound to 0.046 m.e. per gram in the lowest ratio.

The sulfides of arsenic prepared from Na_2S and sodium polysulfide, all possess a moderate base exchange capacity when treated with Ba-acetate at pH 7. In acid solution there appears to be a certain degree of adsorption of sulfide by arsenious sulfide, in which case some of the valences of the sulfide component are free to link up with cations.

TABLE 17

Cation exchange capacities of arsenious sulfides, expressed in milliequivalents per gram of material

RATIO OF SULFIDE TO As_2O_3		MILLIEQUIVALENTS PER GRAM
Using sodium sulfide	Using sodium polysulfide	
m.e.	*m.e.*	
30:25	.....	0.283
25:25	.....	0.268
20:25	.....	0.253
15:25	.....	0.247
10.25	.....	0.278
10:50	.....	0.260
.....	30:25	0.180
.....	30:30	0.216
.....	10:30	0.360



(H)

Sulfides of tin, in which there is a high ratio of sulfide to tin, have a high base exchange capacity at pH 7. It is, in effect, higher than that shown by most inorganic soil colloids which have a high SiO_2/R_2O_3 ratio.

Stannous hydroxide exhibits an appreciable base exchange capacity. When the hydroxide is one-fifth saturated with sulfide ion, this capacity rises from 0.408 m.e. per gram $Sn(OH)_2$ to 0.617 m.e. per gram in the sulfide compound.

Like the highest ratio compounds of ferric ferrocyanide, the highest ratio compounds of stannous sulfide are partially decomposed by hydrolysis at pH 7, hence the two highest ratio compounds which hydrolyze most, have their exchange capacities diminished. Had leaching with Ba-acetate been carried out at a lower pH, the base exchange capacity would undoubtedly have increased with the increasing ratio of sulfide to tin, in a more decided manner.

The sulfides of antimony and bismuth have small base exchange capacities, indicating that at pH 7 there is little hydrolysis of these compounds, with ensuing free valences for linking with cations in solution. In the case of antimony sulfide, there is greater base exchange capacity with increasing saturation of the oxychloride with sulfide, but with bismuth sulfide the base exchange capacities are too small to be significant.

TABLE 18

Cation exchange capacities of stannous sulfides, expressed in milliequivalents per gram of material

RATIO OF TIN TO SULFIDE	MILLIEQUIVALENTS PER GRAM
m.e.	
8:9.6	0.818
8:8.0	0.810
8:6.4	0.951
8:4.8	0.897
8:3.2	0.884
8:1.6	0.617
Isoelectric stannous hydroxide	0.408

TABLE 19

Cation exchange capacity of antimony and bismuth sulfides, expressed in milliequivalents per gram of material

RATIO OF Sb TO S	RATIO OF Bi TO S	MILLIEQUIVALENTS PER GRAM
m.e.	*m.e.*	
16:16	....	0.13
16: 9.6	....	0.089
16: 3.2	....	0.040
....	16:16	0.051
....	16: 9.6	0.036
....	16: 3.2	0.048

GENERAL DISCUSSION

Although the ferric ferrocyanide complex formation and behavior are in general governed by the same principles as the formation and behavior of the amphoteric phosphated, silicated, humated, and proteinated (6) complexes of iron and aluminum, yet they differ from the latter in many ways: (a) All the ferrocyanide, even a certain equivalent excess, is carried down in the precipitate. (b) Small amounts of ferrocyanide cause a great downward displacement of the isoelectric pH of the ferric complex. (c) Except in system 1 there are no colloidal dispersion on the positive side even at low pH and no ionic dispersion, i e., solution. (d) At no very high pH (below the neutral point) is the hydrolysis extensive.

These facts taken together might help us to explain the behavior of this series. The ready hydrolysis, i.e., the ease with which the OH ions displace ferrocyanide ions, shows that the solubility product of the ferric ferrocyanide is relatively high. The fact that the compounds in system 1 and the succeeding ferric ferrocyanide systems are isoelectric at low, and not greatly differing, pH values can therefore not be due to a complete association of the ferrocyanide ions, thus preventing the entrance of dissociable Cl ions. The Cl ions are present in these complexes in greater number than were found in the isoelectric "silicates." The low isoelectric points of the ferric ferrocyanides must be ascribed to the fairly strong acid residue of the ferrocyanide group. This means a high cation dissociation even at low pH. More dissociable Cl is therefore required to render the complex isoelectric, hence the low isoelectric points.

An amphoteric colloid having a strong acid residue must have a correspondingly weaker basic residue because the neutralization must proceed until the product of the H and OH ions equals the ion product of water. The isoelectric pH must then be far below the neutral point because only at such low pH will the dissociation of the weak basoid group balance that of the acidoid group.

Stannous "hydroxide" or oxychloride is isoelectric at about pH 6.6. It has therefore somewhat stronger acidic than basic properties. This accounts for the considerable adsorption and exchange of cations at pH 7. Iron and aluminum "hydroxides" are isoelectric when precipitated from the chlorides by NaOH at about 7.1 and 8.1 respectively. None of these adsorb or exchange cations at the neutral point, but they adsorb large quantities of bases from alkaline solutions. These are therefore stronger as basoids than as acidoids (2).

When the sulfide ions displace the dissociable Cl ions the isoelectric pH is displaced to the acid side: (*a*) because the anionic dissociation of the complex is suppressed and (*b*) because the $-SH$ group introduces a stronger acid residue and thus increases the content and dissociation of diffusible cations. The cation exchange capacity is therefore greater in the sulfide complex.

Let us now briefly consider the three members of the same group; namely, arsenic, antimony, and bismuth.

Arsenic oxide is mainly acidic but the fact that it forms a chloride shows it to be amphoteric. If the compound remained insoluble its particles would undoubtedly be found to have an isoelectric point and to become electropositive at very low pH, since the acidic properties of arsenic are much stronger than the basic. But solution results, and the positive complex resolves itself into single (or an approach to single) ions such as $As_2O_2^{++}$, AsO^+, and As^{+++} and is no longer within the domain of the colloid chemist. The same would be true of of Al and Fe if the isoelectric pH of their hydroxides were below the point of true solution. No positive sols of aluminum and iron hydroxides would then be known.

The fact that arsenious oxide and especially the oxychloride are quite soluble accounts for the uniform composition of the precipitates of the various arsenious sulfide systems and for the nearly constant exchange capacities of these compounds. The formation of a natural series as in the various other systems cannot here take place. Displaceable hydrogen as indicated in formula (*H*) must, however, be assumed.

The oxychloride of antimony is isoelectric at pH 5.6 and is therefore more acidic or less basic than tin but more basic than arsenic. Being considerable stronger as an acidoid than as a basoid, its electropositive and basic properties are completely suppressed by the entrance of small amounts of sulfide ions into the complex. All of the antimony sulfide complexes are therefore negative down to the lowest pH values investigated.

The low exchange capacities of the various electronegative "sulfides" indicate a weak acidoid or a small acid residue. But it must be pointed out that the ion exchange capacity as determined by leaching is never a good criterion since one never knows how far the hydrolysis proceeds.

Bismuth is the most basic element of the three members of the group and we should expect the oxychloride to be isoelectric at a higher pH than the corresponding compound of antimony. The fact that the oxychloride of bismuth is electronegative over the entire range of pH may be due to the Cl ions not being dissociated by the complex. We have selected the Na and Cl ions as the diffusible ions which are most likely to be highly dissociated by the colloidal complexes. Where this is not the case we shall encounter irregularities. Thus in the case of silver chloride, HCl or any other chloride will cause a negative charge (6).

While a positive charge appears to be associated solely with basic materials which combine with acids and dissociate diffusible anions, a negative charge is no indication that the material is an acidoid. All inert substances such as collodion, air bubbles, etc., charge themselves negative in water. This must be a property of the water itself due perhaps to the orientation of the interfacial layer of molecules and to an unequal distribution of the OH and H ions at the phase boundary. The positive charge resulting from the dissociation of anions is therefore not only opposed by the negative charge resulting from the dissociation of cations but also by a certin phase boundary potential. The isoelectric point may therefore not represent that pH at which the dissociation of anions and that of cations are equal, but a somewhat lower pH.

SUMMARY

A series of isoelectric precipitates of "ferric ferrocyanide" and "sulfide of tin" and electronegative "sulfides" of antimony, bismuth, and arsenic, were prepared and their base adsorption and exchange capacities determined.

Details of the method of preparation of the isoelectric compounds, together with flocculation effects, cataphoretic and pH measurements are given.

The introduction of the ferrocyanide ion in increasing amounts into the

oxychloride of iron, results in compounds whose isoelectric points are at low ranges.

Arsenious sulfide is strictly electronegative, and formed under different conditions, its composition always closely approaches a stoichiometric relationship. Arsenic appears to be too acidic to exist as a colloidal cation like the distinctly metallic elements.

A series of isoelectric sulfides of tin, in which the proportion of sulfide and base varied, showed that the isoelectric points of the compounds were at lower pH values, as the compounds became more saturated with sulfide sulfur. When sulfide is in excess of tin, as when ferrocyanide is in excess of iron, no isoelectric point for the compound exists.

The isoelectric point of antimony oxychloride is at a comparatively low pH, despite the high content of chlorine at the isoelectric point. This chlorine appears to be very slightly dissociated. All sulfides of antimony are electronegative.

Bismuth oxychloride represents the extreme condition among the metals under review, in that it has no isoelectric point, being electronegative over a wide pH range.

The base exchange capacity of the ferric ferrocyanides, at a pH where the higher ratio compounds did not decompose, was found to increase with the increasing ratio of the acidoid to the ampholytoid component. The same phenomenon was found in the sulfides of tin and antimony. Arsenious sulfide has a moderate exchange capacity at pH 7, that of the sulfides of bismuth being slight. Further investigations of this type into the behavior of all the metallic elements of the periodic system should bring out some interesting relationships. Work in this direction is now in progress at this institution.

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EQUILIBRIA OF THE BASE-EXCHANGE REACTIONS OF BENTONITES, PERMUTITES, SOIL COLLOIDS, AND ZEOLITES[1]

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It is exceedingly difficult to make accurate base-exchange measurements on soils because of their extreme heterogeneity. This fact has led to the study of the simpler base-exchange substances as a substitute for soil material. Many investigators have studied the synthetic material, called "permutite," under the impression that the results thus obtained would in some measure be applicable to the base-exchange phenomenon of soils. Some soil workers refer to the exchange-complex of soils as "zeolites" or "zeolitic substances." Before any results of experimental studies on permutites, zeolites, or any other base-exchange material can be applied appropriately to soils it must be shown that their identity is complete. It is the purpose of this paper to present, in a summarized manner, a comparison of some of the properties of the four main types of hydrated alumino-silicate base-exchange substances: bentonites, permutites, soil colloids, and zeolites; and to present in detail the preliminary results of the author's attempts at investigating the physical chemistry of the base-exchange reaction for representatives of these substances.

In a recent paper, Kelley, Dore, and Brown (4) present the results of some comparative studies of these base-exchange substances. Their results summarized briefly are:

> The colloidal material of soils and of bentonites contains alkali and alkali-earth bases that are not replaceable, whereas the bases in permutites and moderately ground zeolites are practically completely replaceable. The non-exchangeable bases in bentonites are either magnesium alone, or magnesium and potassium; in soil colloids they are mainly magnesium with some potassium. By prolonged grinding in the ball-mill these non-replaceable bases become exchangeable. Prolonged heating at a temperature of 350°C. does not appreciably lower the exchange capacity of either soil colloids or of bentonites; whereas that of ground natrolite was reduced to 46 per cent, that of stilbite to 7 per cent, and that of scolecite to 6 per cent of the original exchange capacities. X-ray examination showed that permutites are devoid of sufficient crystal structure to give a pattern by the Debye-Scherer method, and that none of the zeolites and feldspars examined gave an X-ray pattern having any resemblance to those of soil colloids. The X-ray patterns of soil colloids were like those of certain of the bentonites. These X-ray results are substantiated by those of Hendricks and Fry (2).

In addition to the findings of Kelley, Dore, and Brown, there are other points of similarity and differences which should be kept in mind. The colloidal properties of bentonites are very similar to those of soil colloids, the calcium-saturated forms being granular and capable of settling out of water. The sodium-saturated forms are highly dispersed in water and on the addition of an electrolyte coagulate to a flocculent mass. The behavior in water of those permutites prepared and studied in this laboratory, is practically uninfluenced by the nature of the exchangeable cation; the sodium-saturated permutites lack the high degree of dispersion in water that bentonites and soil colloids possess. Soil colloids and bentonites are remarkably stable toward dilute acids and alkalies, whereas permutites are easily decomposed by dilute acids and alkalies; and zeolites are also decidedly unstable. A large proportion of both the replaceable and non-replaceable bases of bentonites and soil colloids consists of magnesium; and by simple leaching with a solution of a magnesium salt, bentonites and soil colloids can be prepared with magnesium as the only replaceable base present. In contrast to this, no magnesium-zeolites have as yet been reported; and hitherto it has not been possible to prepare a permutite with more than 50 per cent of the replaceable bases as magnesium, by leaching with magnesium-salt solutions.

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[1] Paper No. 247, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

It does not seem likely that all these differences between soil colloids and bentonites on the one hand and permutites and zeolites on the other are caused by a mere difference in crystal structure. Yet there is a remote possibility that bentonites, permutites, and zeolites are only different crystalline modifications of the same fundamental substance, for they are all hydrated alumino-silicates of fairly similar composition; and it is conceivable that they may all behave similarly as regards the base-exchange reaction. Comparatively little study has been made of the physical chemistry of the cation-exchange reaction. Kerr (5) has investigated the calcium-magnesium exchange reactions of bentonites, permutites, soils, and natural zeolites. His conclusions are that soils and bentonites are very similar, and that zeolites and permutites differ from soils. The writer has been investigating the physical chemistry of the cation-exchange reaction for some representatives of the four principal types of hydrated alumino-silicates, the preliminary results being presented in this paper.

THEORETICAL

The reaction between a solution of potassium chloride and a sodium-saturated base-exchange substance can be represented by the equation

$$\mathrm{NaZ} + \mathrm{K}^+ = \mathrm{KZ} + \mathrm{NA}^+,$$

where Z symbolizes the complex anion of the substance. When this system is given sufficient time to attain equilibrium the conditions of the system are expressed by the equation:

$$\frac{(\mathrm{KZ})}{(\mathrm{NaZ})} \times \frac{(\mathrm{Na}^+)}{(\mathrm{K}^+)} = K \qquad (A)$$

In which K is the equilibrium constant, and all the quantities in parentheses are the activities (7, chap. 22) of the four constituents of the reaction. These activities, or active concentrations, must not be confused with the actual or stoichiometric concentrations. But where, as in this experimental work, the solutions are fairly dilute and of nearly constant ionic strength (7, p. 373) we can use the actual stoichiometric concentrations of sodium and potassium in solution, since the activity coefficients (7, Chap. 28) of sodium chloride and potassium chloride in dilute solutions are nearly equal. Any pure solid taking part in a chemical reaction has an activity equal to unity. For example, in the reaction.

$$BaCO_3 + SO_4^{=} = BaSO_4 + CO_3^{=}$$

the equilibrium conditions are expressed by the relationship

$$K = \frac{CO_3^{=}}{SO_4^{=}}$$

because the $BaSO_4$ and the $BaCO_3$ are present as independent solid phases and the amount present is without influence. Assuming the NaZ and KZ to act like two independently existing solid phases, equation A reduces to

$$\frac{Na^+}{K^+} = K \qquad (B)$$

Kerr (5) has shown that for the calcium-magnesium exchange reaction this equation does not represent even approximately the facts for any of the base-exchange substances which he investigated. My work on the sodium-potassium, calcium-ammonium, and potassium-ammonium exchange reactions confirms his results; so this simple hypothesis may be dismissed.

Kerr (5) has advanced the idea of equating the active mass with the actual amount present in each form. Thus equation A becomes

$$\frac{KZ}{NaZ} \times \frac{Na^+}{K^+} = K \qquad (C)$$

But it must be noted that in equating active with actual concentration the tacit assumptions are made that the two substances NaZ and KZ behave as if in true solution and have the same activity coefficients. The author finds it exceedingly difficult to subscribe to this idea and prefers an entirely different viewpoint. If the NaZ and KZ are capable of forming a continuous series of mixed crystals their activities would no longer be unity. Whenever a substance in a mixed crystal enters into a chemical reaction its activity is not unity but is equal to its molecular fraction in the mixed crystal. Thus the activities of the NaZ and KZ would be given by the equations

$$(NaZ) = \frac{NaZ}{NaZ + KZ}$$

and

$$(\mathrm{KZ}) = \frac{\mathrm{KZ}}{\mathrm{NaZ} + \mathrm{KZ}}$$

respectively. Substitution of these values into equation A leads to the same equation C derived on the basis of Kerr's idea.

Thus far we have assumed that the complex anion, Z, is monobasic. Considering it to be divalent, the reaction would be represented by the expression

$$\mathrm{Na_2Z} + 2\,\mathrm{K^+} = \mathrm{K_2Z} + 2\,\mathrm{Na^+},$$

and the equation representing equilibria conditions, for both the mixed crystal hypothesis and Kerr's idea, would be

$$\frac{\mathrm{K_2Z}}{\mathrm{Na_2Z}} \times \left(\frac{\mathrm{Na^+}}{\mathrm{K^+}}\right)^2 = K \qquad (D)$$

Without presenting any experimental results at this point, suffice it to say that all the data support the monobasic formula and not the dibasic. So, for the sake of brevity we shall omit any further theoretical consideration of a divalent complex anion.

The study of an exchange reaction of two cations of the same valence does not permit one to distinguish between Kerr's theoretical treatment of the two solid phases, NaZ and KZ, and the mixed crystal hypothesis. But the exchange reaction using two dissimilar cations does afford data for distinguishing between the two hypotheses. Using a solution of ammonium chloride and a calcium-saturated material the reaction is

$$2\,\mathrm{NH_4Z} + \mathrm{Ca^{++}} = \mathrm{CaZ_2} + 2\,\mathrm{NH_4^+},$$

and the corresponding expression for the equilibrium conditions in terms of the activities of the four constituents is

$$K = \frac{(\mathrm{NH_4^+})^2}{(\mathrm{Ca^{++}})} \times \frac{(\mathrm{CaZ_2})}{(\mathrm{NH_4Z})^2} \qquad (E)$$

Here again the parentheses denote activities. Since the solutions used in this experimental work are fairly dilute and of nearly constant ionic strength the error introduced by the omission of the activity coefficients of $\mathrm{NH_4Cl}$ and $\mathrm{CaCl_2}$ solutions is nearly constant for all the experiments. Assuming the particles of the substances to be so finely divided as to behave as if in solution (6), equation E becomes

$$K = \frac{\mathrm{NH_4^{+2}}}{\mathrm{Ca^{++}}} \times \frac{\mathrm{CaZ_2}}{\mathrm{NH_4Z^2}} \qquad (F)$$

If the NH_4Z and CaZ_2 form a continuous series of mixed crystals a different expression results. The activities of the two forms of the substance are given by the expressions

$$(NH_4Z) \quad \frac{NH_4Z}{NH_4Z + CaZ_2}$$

and

$$(CaZ_2) = \frac{CaZ_2}{NH_4Z + CaZ_2}$$

Substitution of these values into equation E results in the expression

$$K = \frac{NH_4^{+2}}{Ca^{++}} \times \frac{CaZ_2(CaZ_2 + NH_4Z)}{NH_4Z^2} \qquad (G)$$

which differs from equation F by the factor $(NH_4Z + CaZ_2)$. Thus the data obtained by a study of the equilibria of the calcium-ammonium exchange reaction should afford a means of distinguishing between the two theories as to the rôle of the solid phases.

THE MATERIALS USED AND THEIR PREPARATION

The author studied the sodium-potassium exchange reaction for the following materials: two bentonites, two permutites, two soil colloids, and two zeolites.

The colloidal fractions of the two soils were prepared by sedimentation of the sodium-saturated soil in water; only that material remaining suspended in the upper 20 cm. after a period of 24 hours was used. The suspended material was coagulated by the addition of ammonium chloride. Treatment with ammonium hydroxide solution, followed by oxidation with 5 per cent hydrogen peroxide solution served to remove practically all the organic matter. The use of hydrogen peroxide results in an acid preparation. To remove the hydrogen ions the material was treated with a dilute solution of calcium hydroxide until distinctly alkaline; then it was rendered practically neutral by continued leaching with normal ammonium chloride solution, adjusted to a pH of 6.8 to 7.0 until the ammonium chloride solution came through with its pH unaltered.

The colloidal fractions were separated from two bentonites in the same manner, except that the treatment for the removal of organic matter and the subsequent use of calcium hydroxide solution were omitted because these clays contained little if any organic matter. Bentonite 7 is of the montmorillonite type, having a silica-alumina ratio of very nearly 5 to 1. Bentonite 5 is more like the beidellite type and has a silica-alumina ratio of about 4 to 1.

The two natural zeolites, analcite and heulandite, whose compositions approximated very closely the theoretical, $Na_2O \cdot Al_2O_3 \cdot 4\ SiO_2 \cdot X\ H_2O$ and $CaO \cdot$

$Al_2O_3 \cdot 6\ SiO_2 \cdot X\ H_2O$ respectively, were ground rather finely before being used. They were treated by decantation with neutral normal ammonium chloride solution until most of their cations had been replaced by the ammonium ion.

Two synthetic preparations were investigated. The one, permutite 51, having a composition expressed by the formula $Na_2O \cdot Al_2O_3 \cdot 5\ SiO_2 \cdot X\ H_2O$, was prepared by the addition of hot sodium aluminate solution to silicic acid suspended in boiling water. After being heated on the steam bath for a short time the product was filtered off and leached on a Buchner funnel with neutral normal sodium chloride solution until the leachate came through only slightly alkaline to phenolphthalein. It was then leached with neutral normal ammonium chloride solution until this solution came through unchanged. Permutite 31 had the composition $Na_2O \cdot Al_2O_3 \cdot 3\ SiO_2 \cdot X\ H_2O$; it was prepared by mixing boiling hot solutions of sodium aluminate and sodium silicate. It was then treated exactly as was the other permutite.

TABLE 1

Replaceable bases in bentonites and soil colloids

(Expressed as milligram equivalents per 100 gm.)

MATERIAL	K-SATURATED FORM			Na-SATURATED FORM		
	NH_4 absorbed	Na displaced	K displaced	NH_4 absorbed	Na displaced	K displaced
Bentonite 7	99.7	0.6	97.9	109.8	108.2	0.2
Bentonite 5	86.7	0.0	89.3	93.5	92.1	0.2
Soil colloid 431	67.5	0.6	63.9	69.3	66.4	0.7
Soil colloid 5696	45.8	0.6	46.4	51.3	51.8	0.5

Each of these eight preparations was divided into two nearly equal portions after the treatment with neutral ammonium chloride solution; the one portion was leached on a Buchner funnel with neutral normal sodium chloride solution until the leachate gave no Nessler test for ammonia. The other portion was similarly treated with potassium chloride solution. The products were then freed from all but merest traces of chlorides by leaching with methyl alcohol. They were air-dried and made to pass an 80-mesh sieve.

The replaceable bases in the bentonites and soil colloids were determined by the ammonium acetate method, the absorbed ammonia being determined by aeration. The ammonium acetate leachates were analyzed for sodium and potassium only. All sodium determinations reported in this paper were made by a slight modification of the Barber-Kolthoff method (1). All the potassium determinations were made by the perchloric acid method. The results for the replaceable bases in the bentonite and soil preparations are given in table 1.

The zeolite and permutite preparations were analyzed by the standard methods, and the results are presented in table 2.

TABLE 2

Analyses of permutites and zeolites

SUBSTANCE	PER CENT		M.E. PER 100 GM.		
	SiO_2	Al_2O_3*	Na	K	Ca
Permutite 51, K form	53.64	17.83	3.5	306.8	...
Permutite 51, Na form	53.63	17.92	294.0	0.0	...
Permutite 31, K form	41.62	24.35	1.8	404.0	...
Permutite 31, Na form	44.06	25.61	421.0	0.0	...
Heulandite, K form	56.74	16.56	1.6	283.0	9.7
Heulandite, Na form	58.54	17.38	292.0	0.0	3.7
Analcite, K form†	54.75	22.72	20.0	393.0	...
Analcite, Na form	55.00	22.95	421.0	0.0	...

* These figures include the undetermined but small amounts of Fe_2O_3.

† The X-ray pattern of this potassium-saturated analcite was practically identical with that of leucite.

TABLE 3

Experimental data for bentonite 7, soil colloid 431, and permutite 51, for the sodium-potassium exchange reaction

(All concentrations expressed as milligram equivalents per liter)

INITIAL CONCENTRATIONS				ANALYTICAL RESULTS					
				Bentonite 7		Soil colloid 431		Permutite 51	
Na^+	K^+	NaZ	KZ	Na^+	K^+	Na^+	K^+	Na^+	K^+
100.0	0.0	0.0	25.0	86.3	14.9	87.2	13.9	86.3	14.0
100.0	0.0	0.0	42.9	81.1	20.6	81.8	20.1	80.2	20.2
85.7	14.3	0.0	42.9	71.9	29.2	71.7	29.8	70.0	30.4
71.4	28.6	0.0	42.9	61.8	39.5	61.1	40.8	59.2	41.5
57.1	42.9	0.0	42.9	50.5	50.6	49.5	52.0	47.7	53.0
42.9	57.1	0.0	42.9	38.7	62.2	37.7	64.4	35.3	65.2
25.0	75.0	0.0	25.0	23.7	76.9	23.4	78.1	21.9	78.5
75.0	25.0	25.0	0.0	85.3	15.6	86.3	14.9	86.2	14.9
57.1	42.9	42.9	0.0	79.8	22.3	80.7	21.8	78.0	21.8
42.9	57.1	42.9	0.0	70.4	31.5	70.4	31.9	67.8	32.3
28.6	71.4	42.9	0.0	60.0	42.1	59.7	42.8	57.0	43.2
14.3	85.7	42.9	0.0	48.4	53.1	48.4	54.1	46.0	54.5
0.0	100.0	42.9	0.0	37.0	64.7	36.7	65.7	34.1	66.2
0.0	100.0	25.0	0.0	22.7	78.3	22.9	78.6	21.2	78.8

EXPERIMENTAL PROCEDURE

The sodium-potassium exchange reaction was experimentally investigated at a temperature of 25°C. and at a total concentration of tenth-normal. In order to approach equilibrium in both directions two series of experiments were carried out simultaneously with most of the materials. In the one series various amounts of NaZ were used with 50 cc. of a tenth-normal solution containing varying ratios of sodium and potassium chlorides. In the other series of experiments corresponding amounts of KZ were used with 50 cc. of a tenth-normal solution of sodium and potassium chlorides in varying ratios. The ratio of sodium to potassium in the entire system varied from about 5:1 down to 1:5, thereby covering the composition range about as completely as the analytical accuracy permitted. The weighed amounts of material and 50 cc. of the

TABLE 4

Experimental data for heulandite, permutite 31, and soil colloid 5696

(All concentrations expressed as milligram equivalents per liter)

INITIAL CONCENTRATIONS				ANALYTICAL RESULTS					
				Heulandite		Permutite 31*		Soil colloid 5696	
Na^+	K^+	NaZ	KZ	Na^+	K^+	Na^+	K^+	Na^+	K^+
100	0	0	20	90.9	8.9	87.7	11.8	90.1	10.9
100	0	0	30	88.2	11.9	83.8	15.9	87.2	14.5
100	0	0	40	85.6	14.2	80.5	19.5	84.7	17.1
100	0	0	50	83.7	16.5	77.8	22.5	82.4	19.7
80	20	0	50	69.6	30.6	63.5	36.8	68.7	33.1
70	30	0	50	62.0	38.5	56.0	44.6	61.1	41.1
50	50	0	50	45.1	55.7	40.8	60.8	44.6	57.1
40	60	0	40	37.0	63.9	33.7	67.2	36.6	64.8
30	70	0	30	27.9	72.2	26.4	74.8	28.3	72.9
20	80	0	20	19.43	81.3	18.4	82.5	19.3	81.5
80	20	20	0	90.7	9.1	86.7	13.0	89.1	11.6
70	30	30	0	87.8	12.2	81.9	17.9	86.0	15.5
60	40	40	0	84.9	14.7	77.5	21.9	83.4	18.7
50	50	50	0	82.5	17.5	74.6	25.9	80.9	21.2
30	70	50	0	69.0	31.6	60.1	40.4	67.0	35.2
20	80	50	0	61.2	39.5	52.0	47.7	59.7	42.8
0	100	50	0	44.6	56.1	37.8	64.4	43.5	58.9
0	100	40	0	36.3	63.9	31.3	69.9	35.7	65.8
0	100	30	0	27.7	72.0	24.6	76.3	27.6	73.8
0	100	20	0	19.32	81.3	17.1	83.7	18.9	81.9

* As a result of an inadvertent error, the actual amounts of sodium-saturated permutite 31 used are 2.4 per cent less than the values given.

proper sodium-potassium chloride solutions were put into pyrex test tubes, stoppered well with rubber stoppers, and left in the 25°C. thermostat from 7 to 14 days. The test tubes and contents were frequently shaken. The solutions were then filtered off and the sodium and potassium present in the filtrates were determined analytically. The experimental data showing the amounts of NaZ or KZ and the compositions of the sodium-potassium chloride solutions used, together with the analytical results, are given in tables 3, 4, and 5.

EXPERIMENTAL RESULTS AND DISCUSSION

Bentonite 7, permutite 51, and soil colloid 431 were investigated by exactly the same procedure. The initial amounts of NaZ, KZ, NaCl, and KCl were the same for all three substances; these data are given in table 3 along with the analytical results.

The proportions of the four constituents were slightly altered in the experiments with the rest of the base-exchange materials. The data for the initial concentrations and the analytical results for heulandite, permutite 31, and soil colloid 5696 are given in table 4.

TABLE 5

Experimental data for bentonites 5 and 7, soil colloid 431, and analcite

(All concentrations expressed as milligram equivalents per liter)

INITIAL CONCENTRATIONS				ANALYTICAL RESULTS							
				Bentonite 5		Bentonite 7		Soil colloid 431		Analcite	
Na^+	K^+	NaZ	KZ	Na^+	K^+	Na^+	K^+	Na^+	K^+	Na^+	K^+
80	20	20	0	88.8	11.6	87.4	13.5	....	....	....	....
70	30	30	0	....	....	....	....	84.7	17.4	73.3	27.2
30	70	50	0	66.3	34.9	64.8	37.9	....	....	....	....
20	80	50	0	....	....	....	....	57.8	45.7	29.1	71.2
0	100	40	0	....	....	34.9	67.0	....	....	....	....
0	100	30	0	27.5	73.7	....	....	27.3	75.3	8.3	92.6

TABLE 6

Equilibria constants for bentonite 7 for the reaction $NaZ + K^+ = KZ + Na^+$

(Equilibrium approached from the right)

CONCENTRATIONS IN M.E. PER LITER				EQUILIBRIUM CONSTANTS		
Na^+	K^+	NaZ	KZ	Equation B	Equation C	Equation D
86.3	14.9	13.7	10.1	5.8	4.3	25.0
81.1	20.6	18.9	22.3	3.9	4.6	18.0
71.9	29.2	13.8	28.0	2.5	5.0	12.5
61.8	39.5	9.6	31.9	1.6	5.2	8.3
50.5	50.6	6.6	35.1	1.0	5.3	5.3
38.7	62.2	4.2	37.8	0.6	5.6	3.5
23.7	76.9	1.3	23.1	0.3	5.5	1.7

Only three experiments were made with bentonite 5 and with analcite; a few experiments with soil colloid 431 and bentonite 7 were repeated to arrive at some idea of the reproducibility of the results. The data for these experiments are given in table 5.

Calculations based on the data for bentonite 7, given in tables 3 and 5, are presented in tables 6 and 7. The first two columns give the analytical results for the milligram equivalents of sodium and potassium per liter of the solution.

The third and fourth columns give the calculated values for the concentrations of the NaZ and KZ as milligram equivalents per liter in the equilibria mixtures; these values are obtained by subtracting the sodium or potassium, found in the solution, from the total amounts present in the system. The figures in the fifth column are the values of the equilibrium constant K calculated according to equation B, those in the sixth column are the values for equation C, and those in the seventh column are the values for equation D. The values of the calculated equilibria constants in the fifth column of tables 6 and 7 show that the two solid forms NaZ and KZ cannot be treated like two independent solid phases. The values in columns six and seven indicate strongly that the complex-anion is monobasic rather than dibasic. The calculated values for the equilibria constants for the other substances show the same general trend.

TABLE 7

Equilibria constants for bentonite 7 for the reaction $NaZ + K^+ \quad KZ + Na^+$

(Equilibrium approached from the left)

CONCENTRATIONS IN M.E. PER LITER				EQUILIBRIUM CONSTANTS		
Na^+	K^+	NaZ	KZ	Equation B	Equation C	Equation D
85.3	15.6	14.7	9.3	5.5	3.5	19.3
79.8	22.3	20.2	20.6	3.6	3.6	13.0
70.4	31.5	15.3	25.6	2.2	3.7	8.1
60.0	42.1	11.4	29.3	1.4	3.7	5.2
48.4	53.1	8.7	32.6	0.9	3.4	3.0
37.0	64.7	5.9	35.3	0.6	3.4	2.0
22.7	78.3	2.3	21.7	0.3	2.7	0.8
87.4	13.5	12.6	6.5	6.5	3.3	21.0
64.8	39.7	15.2	32.1	1.6	3.6	5.9
34.9	67.0	5.1	33.0	0.5	3.4	1.8

It will be noted that the figures in column six of tables 6 and 7 give a different value for the "equilibrium constant," depending on the direction in which equilibrium is approached. The difference is such as to indicate that insufficient time was allowed for the attainment of true equilibrium. However, experimental evidence, to be presented later, points strongly to some other cause for this so-called "hysteresis." All of the materials, for which the equilibria of the sodium-potassium exchange reaction were approached in both directions, exhibited this hysteresis and approximately to the same degree.

Rather than further to burden this paper with numerous tables of calculations the results are presented graphically in figure 1. The sodium-potassium exchange reaction can be represented by the general equation

$$Na_nZ + nK^+ = K_nZ + nNa^+$$

in which n is the valence or basicity of the complex anion Z. The expression for the equilibrium constant is

$$\frac{K_nZ}{Na_nZ} \times \frac{Na^{+n}}{K^{+n}} \quad K$$

Rearranging the terms and taking logarithms leads to the equation

$$\log\left(\frac{NaZ}{KZ}\right) = n \log\left(\frac{Na^+}{K^+}\right) - \log K$$

This is in the form of the equation for a straight line whose slope is n, and the intercept on the axis of ordinates is the logarithm of the reaction constant K. All the experimental data for the sodium-potassium exchange reaction, in which the sodium-saturated materials were used, are plotted in figure 1. The quantities $\log\left(\frac{NaZ}{KZ}\right)$ are the ordinates and $\log\left(\frac{Na^+}{K^+}\right)$ are the abscissas. The results for analcite fall outside the area of figure 1. Figure 1 shows the great similarity in behavior of soil colloid 431 and bentonite 7, and also the similarity of soil colloid 5696 and bentonite 5. The graphs for these four substances have approximately a slope of unity, indicating the monobasicity of the complex anion. It is seen that heulandite and the two permutites differ noticeably from the soil colloids and the bentonites, and that their graphs have a slope consider-

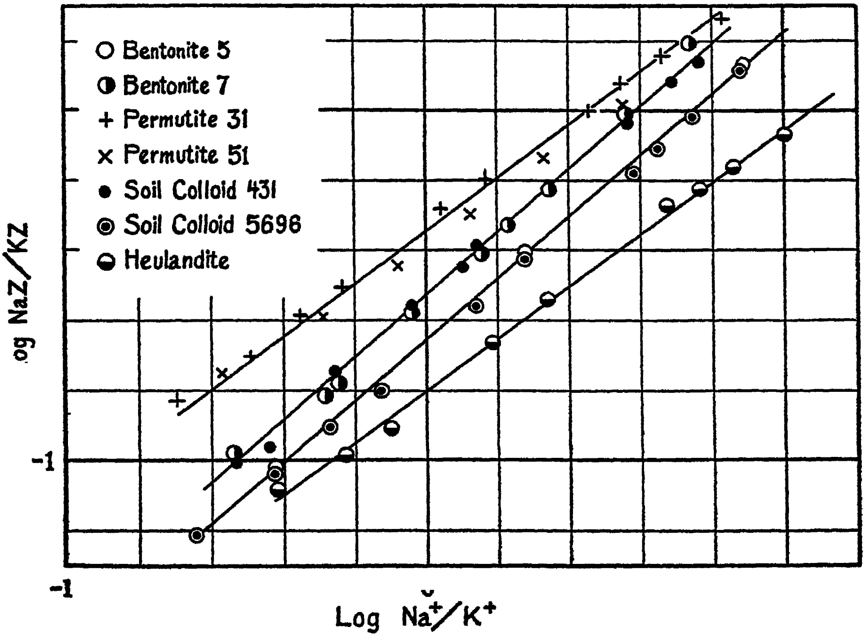

FIG. 1. EQUILIBRIA IN THE SODIUM-POTASSIUM EXCHANGE REACTION OF THE VARIOUS ALUMINO-SILICATES

ably less than unity. This is likewise true for analcite. The apparent valency of less than one for the complex anion of the permutites and zeolites may have its origin in the incomplete validity of the assumption of a continuous series of perfect solid solutions or mixed crystals. Where, as in the case of permutites and zeolites, all of the cation is replaceable this assumption may not be valid, but may be approximately true when only one-fourth to one-third of the total cation content is replaceable. Further work is in progress regarding this point.

The results for the reverse reaction, in which the potassium-saturated materials were used, are not shown in figure 1. They form a similar set of lines all displaced to the right by about the same amount; this is because in no case was true equilibrium attained and all of the base-exchange substances showed about the same amount of hysteresis.

TABLE 8

Experimental data for bentonite 7 for the reaction $2\ NH_4Z + Ca^{++} = CaZ_2 + 2\ NH_4^+$, *equilibrium approached from the left*

(Concentrations expressed as milligram equivalents per liter)

INITIAL CONCENTRATIONS				FINAL CONCENTRATIONS				EQUILIBRIUM CONSTANTS		
				By analysis		Calculated		Dibasic anion	Monobasic anion	
NH_4Z	CaZ_2	NH_4^+	Ca^{++}	NH_4^+	Ca^{++}	NH_4Z	CaZ_2		Equation F	Equation G
20.0	0.0	0.0	100.0	16.8	84.3	3.2	15.7	16	5.4	55
40.0	0.0	0.0	100.0	27.4	73.2	12.6	26.8	21	1.7	44
60.0	0.0	0.0	100.0	36.3	64.7	23.7	35.3	31	1.3	54
80.0	0.0	0.0	100.0	44.1	57.7	35.9	42.3	40	1.1	63
100.0	0.0	0.0	100.0	51.0	51.4	49.0	48.6	49	1.0	73
80.0	0.0	20.0	80.0	55.8	45.4	44.2	34.6	53	1.2	74
60.0	0.0	40.0	60.0	62.1	38.7	37.9	21.3	57	1.5	73
40.0	0.0	60.0	40.0	70.9	29.5	29.1	10.5	61	2.1	72
20.0	0.0	80.0	20.0	83.2	16.8	16.8	3.2	79	4.7	86

INVESTIGATION OF THE RÔLE OF THE SOLID PHASE

In the theoretical section of this paper it was shown that the rôle of the solid phases in the base-exchange reaction could be ascertained only by the use of a reaction involving two cations of dissimilar valences. For this purpose, a series of experiments were performed in which bentonite 7 was used in the calcium-ammonium exchange reaction. These experiments were conducted similarly to those for the sodium-potassium reaction at a total concentration of tenth-normal and a temperature of 25° C. Two series of experiments were carried out, the one using calcium-saturated bentonite 7 and the other using ammonium-saturated bentonite 7; thus equilibrium was approached in both directions. The equilibrated mixtures were filtered, and the filtered solutions were analyzed for both calcium and ammonium. The final amounts of CaZ_2 and NH_4Z present in the mixtures were calculated as the difference between the total amounts of calcium and ammonium in the system and that found in the solution. The experimental data are presented in tables 8 and 9.

In column 9, tables 8 and 9, are given the values for a dibasic complex anion; the values are the same for both the mixed crystal hypothesis and for Kerr's method of dealing with the solid phases. Column 10 gives the values for the equilibrium constant for a monobasic anion calculated by the use of equation F, Kerr's equation. The last column contains the constant for a monobasic anion calculated by the use of equation G for the mixed crystal hypothesis.

It is evident that equation G gives a very much better representation of these experimental data than does Kerr's equation; thus it seems probable that for this particular bentonite the partial substitution of one cation for another produces a mixed crystal or solid solution rather than two solid phases behaving either as independent phases or as if in solution.

TABLE 9

Experimental data for bentonite 7 for the reaction 2 $NH_4Z + Ca^{++} = CaZ_2 + 2\ NH_4^+$*, equilibrium approached from the right*

(Concentrations expressed as milligram equivalents per liter)

INITIAL CONCENTRATIONS				FINAL CONCENTRATIONS				EQUILIBRIUM CONSTANTS		
				By analysis		Calculated		Dibasic anion	Monobasic anion	
NH_4Z	CaZ_2	NH_4^+	Ca^{++}	NH_4^+	Ca^{++}	NH_4Z	CaZ_2		Equation F	Equation G
0.0	20.0	20.0	80.0	17.6	82.4	2.4	17.6	28	11.5	130
0.0	40.0	40.0	60.0	31.8	68.2	8.2	31.8	57	7.0	169
0.0	60.0	60.0	40.0	42.5	57.8	17.5	42.2	75	4.3	166
0.0	80.0	80 0	20.0	51.4	49.7	28.6	50.3	94	3.3	177
0.0	100.0	100.0	0.0	57.6	43.7	42.4	56.3	102	2.4	169
0.0	80.0	100.0	0.0	62.8	39.1	37.2	40.9	112	3.0	173
0.0	60.0	100.0	0.0	67.8	33.1	32.2	26.9	116	3.6	164
0.0	40.0	100.0	0.0	74.7	25.7	25.3	14.3	122	4.8	156
0.0	20.0	100.0	0.0	85.2	15.5	14.8	4.5	142	9.6	163

By taking the logarithms of equations F and G they can be written in the forms

$$\log\left(\frac{Ca^{++}}{NH_4^{+2}}\right) = \log\left(\frac{CaZ_2}{NH_4Z^2}\right) - \log K \qquad (H)$$

and

$$\log\left(\frac{Ca^{++}}{NH_4^{+2}}\right) = \log\left[\frac{CaZ_2(CaZ_2 + NH_4Z)}{NH_4Z^2}\right] - \log K \qquad (I)$$

respectively. Both of these equations are in the form of the straight line equation with a slope of unity. The data in table 9 are plotted as the solid circles in figure 2 according to equations H and I; the quantities $\log\left(\frac{Ca^{++}}{NH_4^{+2}}\right)$ are the ordinates, and the quantities $\log\left(\frac{CaZ_2}{NH_4Z^2}\right)$ and $\log\left[\frac{CaZ_2\,(CaZ_2+NH_4Z)}{NH_4Z^2}\right]$ are the abscissas. The curve on the left is the one for equation H and the one on the right is for equation I. Figure 2 shows graphically what was brought out in tables 8 and 9, that the mixed crystal hypothesis offers a better explanation of the behavior of this bentonite than does Kerr's equation. Both the lines in figure 2 are drawn with the theoretical slope of unity, and the experimental points should fall on or near the line. This they do when plotted according to the mixed crystal equation, but do not when plotted according to Kerr's equation.

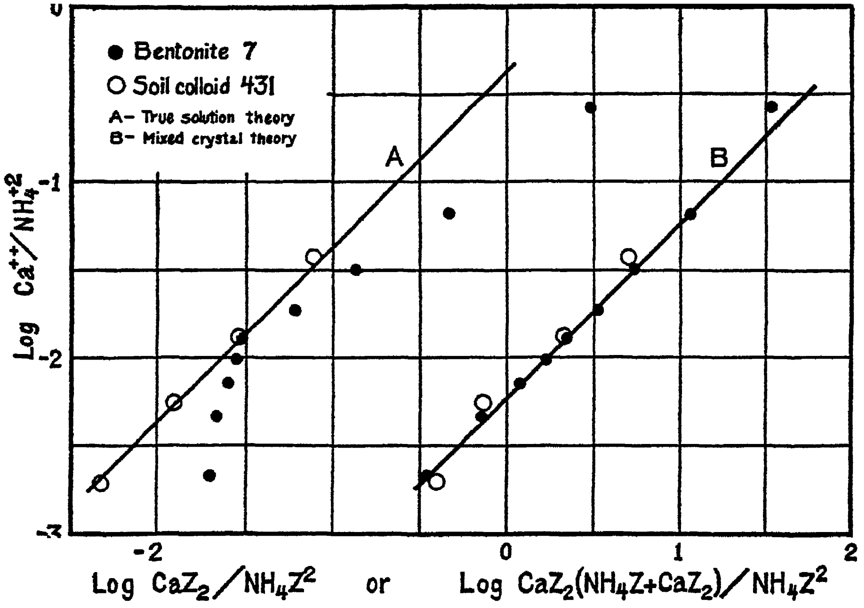

FIG. 2. EQUILIBRIA IN THE CALCIUM-AMMONIUM EXCHANGE REACTIONS OF BENTONITE 7 AND SOIL COLLOID 431

The open circles in figure 2 represent a few somewhat similar experiments with soil colloid 431 and are plotted in the same manner as the bentonite data. The experimental data for these experiments with soil colloid 431 are given in table 10.

These experiments with soil colloid 431 were performed at a temperature of 25°C., the solutions were analyzed for both calcium and ammonium; and the quantities CaZ_2 and NH_4Z were calculated by difference. It will be noted that a constant amount (100 m. e. per liter) of the material was used in these experiments, and for this reason there is little to choose between the two hypotheses; the constants of both equations F and G are fairly uniform, those calculated by Kerr's equation being perhaps a shade more constant. But it is only by varying the amount of bentonite used that the inadequacy of Kerr's formula is brought out. The equation for the equilibrium constant derived on the assumption of mixed crystal formation brings the data for soil colloid 431 into accord with that of bentonite 7. In contrast to this, only one of the bentonite 7 points (the one in which 100 m.e. per liter were used) falls on the line passing through the soil colloid 431 data when plotted according to equation H. The applicability of any theoretically derived formula should not be limited by the necessity of using a constant amount of base-exchange material, as Kerr's equation obviously is.

Kerr (6) studied the calcium-ammonium exchange reaction for an oxidized Hawaiian soil. He used the same amount of soil throughout his series of experiments, consequently his data give constant values for the equilibria constants when substituted into his equation. His data, when substituted into the mixed crystal equation, give a value of about 75 for the reaction constant, as compared to a value of about 170 for bentonite 7 and soil colloid 431. This difference is in all probability due to the fact that Kerr used a different soil from those used here; although other factors, such as differences in temperature and concentrations, no doubt also contribute to the difference. The investigation of the sodium-potassium exchange reaction showed that there are at least two types of soils and bentonites from the standpoint of the physical chemistry of the exchange reaction.

TABLE 10

Experimental data for soil colloid 431 for the reaction $2\ NH_4Z + Ca^{++} = CaZ_2 + 2\ NH_4^+$, *equilibrium approached from the right*

(Concentrations expressed as milligram equivalents per liter)

INITIAL CONCENTRATIONS				FINAL CONCENTRATIONS				EQUILIBRIUM CONSTANTS	
NH_4Z	CaZ_2	NH_4^+	Ca^{++}	NH_4^+	Ca^{++}	NH_4Z	CaZ_2	Equation F	Equation G
0.0	100.0	69.8	30.1	40.2	61.4	29.6	68.7	2.06	132
0.0	100.0	99.8	0.0	57.2	43.6	42.6	56.4	2.29	162
30.0	70.0	99.8	0.0	72.7	29.4	57.1	40.6	2.24	173
60.0	40.0	99.8	0.0	87.3	15.1	72.5	24.9	2.19	183

HYSTERESIS OF THE EXCHANGE REACTION

The phenomenon of hysteresis, or the failure to attain a true equilibrium, was investigated more fully. By using equivalent amounts (20 m.e. per liter) of calcium-saturated bentonite 7 and ammonium chloride solution, and ammonium-saturated bentonite 7 with calcium coloride solution, equilibrium for the reaction

$$2\,NH_4Z + Ca^{++} = CaZ_2 + 2\,NH_4^+$$

was approached in both directions. One set of experiments was performed at room temperature with only 5 hours elapsing before the solutions were filtered off for analyses. The other set of experiments was given frequent shaking for 7 days at a temperature of 75°C. The experimental data are presented in table 11. The solutions were analyzed for calcium and ammonium; the quantities CaZ_2 and NH_4Z were calculated by difference and the equilibrium constants by the use of equation G. The ratio of the values for the "equalibria constants," approached in opposite directions is given in the last column. It is evident from the values of this ratio, that the approach to a true equilibrium

TABLE 11

Effect of time and temperature on the attainment of equilibrium in the reaction $2\,NH_4Z + Ca^{++} = CaZ_2 + 2\,NH_4^+$

(Concentrations expressed in milligram equivalents per liter)

INITIAL CONCENTRATIONS				FINAL CONCENTRATIONS				EQUILIBRIUM CONSTANTS	RATIO OF EQUILIBRIUM CONSTANTS
NH_4Z	CaZ_2	NH_4^+	Ca^{++}	NH_4^+	Ca^{++}	NH_4Z	CaZ_2		
5 hours shaking at room temperature									
19.68	0.0	0.0	20.06	12.68	7.47	7.00	12.59	73	0.39
0.0	20.00	20.12	0.0	14.37	5.91	5.75	14.09	190	
7 days shaking at a temperature of 75°C.									
19.68	0.0	0.0	20.06	14.51	5.84	5.17	14.22	237	0.45
0.0	20.00	20.12	0.0	15.90	4.80	4.22	15.20	530	

is not significantly better in the experiments conducted at 75°C. for 7 days than in those at room temperature for only 5 hours. If it were a question of rate of reaction and sufficient time for the attainment of equilibrium, one would expect a much closer approach to a true equilibrium in those experiments conducted at a temperature of 75°C., at which temperature the rate of reaction would probably be 100 or so times greater. Furthermore, a similar set of experiments which were kept at a temperature of 75°C. for 2 days and then cooled and allowed to remain at room temperature for 5 days gave results almost identical with those obtained in the experiments conducted at room temperature (table 11). The values for the reaction constants were 90 and 200, giving a ratio of 0.45. That experiment in which ammonium-saturated bentonite and calcium chloride solution were used had, on being heated to 75°C., reacted to a greater extent than the system calcium-saturated bentonite and ammonium chloride solution does at room temperature; and yet on cooling and standing at room temperature, little if any effect of the higher temperature remained.

This phenomenon of hysteresis is not confined to bentonites, but occurs also in the exchange reactions of soils, permutites, and zeolites, as has already been mentioned. Neither is it true that all exchange reactions of one substance show this hysteresis. There is little indication of hysteresis in the reaction

$$BaZ_2 + Ca^{++} = CaZ_2 + Ba^{++}$$

as the data presented in table 12 show. These experiments were conducted at 40°C. for 2 days. The solutions were analyzed for both barium and calcium; the quantities BaZ_2 and CaZ_2 were calculated by difference. Similarly, the reaction

TABLE 12

Experimental data for bentonite 7 for the reaction $BaZ_2 + Ca^{++} = CaZ_2 + Ba^{++}$

(Concentrations expressed as milligram equivalents per liter)

INITIAL CONCENTRATIONS				FINAL CONCENTRATIONS				EQUILIBRIUM CONSTANTS	RATIO OF EQUILIBRIUM CONSTANTS
BaZ_2	CaZ_2	Ba^{++}	Ca^{++}	Ba^{++}	Ca^{++}	BaZ_2	CaZ_2		
20.00	0.0	0.0	20.05	9.58	10.50	10.42	9.55	0.837	0.98
0.0	20.00	19.86	0.0	9.54	10.41	10.32	9.59	0.851	

TABLE 13

Experimental data for bentonite 7 for the reaction $BaZ_2 + Cu^{++} = CuZ_2 + Ba^{++}$

(Concentrations expressed as milligram equivalents per liter)

INITIAL CONCENTRATIONS				FINAL CONCENTRATIONS				EQUILIBRIUM CONSTANTS	RATIO OF EQUILIBRIUM CONSTANTS
BaZ_2	CuZ_2	Ba^{++}	Cu^{++}	Ba^{++}	Cu^{++}	BaZ_2	CuZ_2		
20.00	0.0	0.0	19.98	9.38	10.64	10.62	9.34	0.775	0.98
0.0	20.00	19.87	0.0	9.35	10.61	10.52	9.39	0.787	

$$BaZ_2 + Cu^{++} = CuZ_2 + Ba^{++}$$

shows little if any indication of hysteresis; the experimental data for this reaction are presented in table 13. These experiments were conducted at 40°C. for only 3 hours. The final solutions were analyzed for both copper and barium. The final quantities of BaZ_2 and CuZ_2 were calculated by difference.

Kerr (5) studied the calcium-magnesium exchange reaction; and in the case of the Miami silt loam equilibrium was approached in both directions with no evidence of any hysteresis for this reaction.

Hisschemöller (3) studied the reaction

$$NH_4Z + Na^+ = NaZ + NH_4^+$$

for permutites and found very marked hysteresis. By repeatedly transforming the permutite back and forth from a sodium-saturated to an ammonium-saturated condition at room temperature he found that the hysteresis was removed. The author subjected some bentonite 7 to 5 complete calcium-ammonium transformations and then investigated the calcium-ammonium exchange reaction for this material, but found no closer approach to a true equilibrium.

The calcium-ammonium exchange reaction for soil colloid 431 was investigated in the same manner, the data being given in table 14. These experiments were conducted at room temperature for a period of 24 hours. The final solutions were analyzed for both calcium and ammonium; the final quantities of CaZ_2 and NH_4Z were calculated by difference and the constants by equation G. The results are in fair agreement with the room temperature values of bentonite 7.

This phenomenon of hysteresis may possibly be related to some phase of the crystal structures of the base-exchange substances. The fact that none of the exchange reactions involving two divalent cations showed appreciable hysteresis in the case of the bentonite 7 seems of significance to the author, especially when one remembers that this bentonite contains only divalent cations. The author, however, offers no explanation of this phenomenon.

TABLE 14

Experimental data for soil colloid 431 for the reaction $2\ NH_4Z + Ca^{++} = CaZ_2 + 2\ NH_4^+$

(Concentrations expressed as milligram equivalents per liter)

INITIAL CONCENTRATIONS				FINAL CONCENTRATIONS				EQUILIBRIUM CONSTANTS	RATIO OF EQUILIBRIUM CONSTANTS
NH_4Z	CaZ_2	NH_4^+	Ca^{++}	NH_4^+	Ca^{++}	NH_4Z	CaZ_2		
19.40	0.0	0.0	19.93	12.15	8.09	7.25	11.84	54	0.35
0.0	20.00	19.98	0.0	13.98	6.29	6.00	13.71	153	

SUMMARY

The author realizes that this work can be considered only as being of a preliminary nature, as too few soil colloids, bentonites, and zeolites have been investigated to draw any sweeping conclusions. But the few results thus far obtained confirm the conclusions of other investigators that the base-exchange constituents of soils and bentonites are remarkably similar, and that permutites and zeolites differ from the base-exchange complex of soils in many respects.

The investigation of the sodium-potassium exchange reactions of bentonites and soil colloids showed that there are at least two and very probably more classes of bentonites and soil colloids. The results further indicate that the base-exchange complex anions of bentonites and soils are monobasic, in harmony with the work of Kerr.

The results of the experimental investigation of the calcium-ammonium exchange reactions of a bentonite and a soil colloid can best be interpreted by the hypothesis of the formation of a mixed crystal when one replaceable cation is partially replaced by another cation.

The sodium-potassium and the calcium-ammonium exchange reactions of all the materials that were studied showed pronounced hysteresis, or failure to attain "true" equilibria. An attempt to remove, by repeated transformation, the hysteresis in the calcium-ammonium exchange reaction of a bentonite failed to reduce it appreciably. On the other hand, in the case of the bentonite, none of the exchange reactions involving two divalent cations showed any appreciable amount of hysteresis. Experimental evidence indicates that it probably is not a question of rate of reaction or insufficient time for the attainment of equilibrium, but is probably a phenomenon associated with the crystal structure of the alumino-silicates.

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DEHYDRATION, SOIL ACIDITY, AND EXCHANGEABLE BASES*

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In a previous communication the authors have discussed the effect of heat and dehydration upon the acidity of suspensions of soil, and have shown that the increased acidity observed is accompanied by a diminution of the content of exchangeable bases, and an increase in the amount of those present in the solution in contact with the soil (3).

The object of the work which is contained in the present paper is to obtain further information upon the extent of these changes, and to trace the effect of these changes upon the associated anion.

EXPERIMENTAL METHODS

The method employed in measuring the pH of the soils was that described by Bülman (2). A list of the soils employed together with their mechanical analyses, which were worked out under the new international method, is given in table 1.

INVESTIGATION OF THE EFFECT OF HEATING UPON THE EXCHANGEABLE AND WATER-SOLUBLE BASES OF SOILS

Previous experiments showed that the total exchangeable bases in soils were less after heating the soil in a steam oven than in the original soil. The total bases in that work were estimated by the acid neutralization method (3).

In the present work the bases were estimated individually. The soils were leached with normal ammonium chloride and also with water. The ammonium chloride extract will contain the exchangeable bases, plus the water-soluble bases, so that the difference between the bases in the ammonium chloride extract and those in the water extract gives the amount of exchangeable bases. The experimental details were as follows:

In every case, 30 gm. of the damp soil and 25 gm. of the soil previously heated for 48 hours in the oven were used. The soil was weighed out into a beaker and 100 cc. of hot normal ammonium chloride solution added and the mixture was left to stand with occasional stirring overnight. At the same time another exactly similar experiment was performed using water instead of ammonium chloride. In all cases the ammonium chloride and water leachings were done side by side. After standing overnight the liquid was decanted through a filter and the soil was washed on to the filter with ammonium chloride and water respectively. The leaching was then continued until the volumes of the respective filtrates were exactly 1 liter. The bases calcium, magnesium, potassium, sodium, were then estimated in the ammonium chloride and water extracts, and the results expressed as milligram equivalents of base per 100 gm. of dried soil.

In the case of the soil 4, which contains calcium carbonate, a similar leaching with normal sodium chloride, instead of ammonium chloride, was performed for the estimation of the calcium.

TABLE 1

Mechanical analysis of the soils

ORIGIN AND DESCRIPTION OF SOILS	LOSS ON IGNITION	COARSE SAND	FINE SAND	SILT	CLAY	CALCIUM CARBONATE
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1. Downwash from plateau gravel on Kimeridge clay over Coral rag, forest soil	13.27	35.49	12.38	10.85	26.30	....
2. Downwash from plateau gravel on Kimeridge clay over Coral rag, forest soil	13.00	7.32	33.70	20.35	28.42	....
3. Kimeridge clay, pasture soil	10.67	13.67	20.94	22.16	33.42	....
4. Middle lias calcareous ironstone, arable loam	10.59	14.72	26.88	24.00	27.02	0.06
5. Kimeridge clay forest subsoil	4.78	17.33	22.34	19.05	34.81	....
6. Red loam soil from Devon	7.82	24.10	25.23	21.35	22.95	....
7. Fen soil from Peterboro District, Northants	29.93	9.08	11.70	17.33	35.87	....
8. Soil from edge of fens, Cambridge	10.15	0.26	15.42	28.30	47.57	1.19
9. Soil from edge of fen, Cambridge	14.14	0.56	26.94	23.77	36.25	0.35
10. Laterite from Sierra Leone, W. Africa	14.95	29.08	6.98	11.60	48.40	....
11. Ibadakuli soil, Tanganyika, E. Africa	3.93	47.15	23.56	5.60	21.65	....
12. Kondoa-Irangi Dodoma, Tanganyika, E. Africa	5.95	14.76	11.04	16.65	57.75	....
13. Peat from Co. Wicklow, Ireland	.....	......	.....	.....	.....	....
14. Kimeridge clay, heavy pasture soil	14.25	2.62	6.20	17.54	60.72	....
15. Old red sandstone, clay subsoil, Hereford	2.57	3.53	23.38	47.87	23.00	....
16. Old red sandstone, clay subsoil, Hereford	4.13	1.17	15.06	51.27	32.42	....

The calcium was estimated by precipitation as oxalate and titrating with permanganate the oxalic acid formed by the action of sulfuric acid upon this. The magnesium was estimated by precipitation as phosphate and weighing, the potassium by weighing as perchlorate, and the sodium by weighing as sulfate. The results are given in table 2 and show clearly that the amounts of bases present in the ammonium chloride extracts are the same before and after heating, but that there are more water-soluble bases after heating than there were before. Consequently there are less exchangeable bases in the soil after the drying, thus confirming the previous work. Further, these results indicate that the heating has the effect of changing some of the exchangeable bases into water-soluble bases.

TABLE 2

Effect of 48 hours' heat on the soils

Results expressed in milligram-equivalents per 100 gm. dry soil

BASE	BEFORE HEATING			AFTER HEATING		
	NH_4Cl extract	Water extract	Exchangeable bases	NH_4Cl extract	Water extract	Exchangeable bases
Soil 1						
Calcium	39.46	1.72	37.74	39.44	3.12	36.32
Magnesium	2.72	0.21	2.51	2.76	0.86	1.90
Potassium	0.81	0.29	0.52	0.81	0.35	0.46
Sodium	1.91	0.48	1.43	1.93	0.65	1.28
Total	44.90	2.70	42.20	44.94	4.98	39.96
Soil 2						
Calcium	34.72	1.17	33.55	34.65	2.74	31.91
Magnesium	2.26	0.08	2.18	2.19	0.18	2.01
Potassium	0.72	0.17	0.55	0.76	0.43	0.33
Sodium	3.14	1.40	1.74	3.20	1.85	1.35
Total	40.84	2.82	38.02	40.80	5.20	35.60
Soil 3						
Calcium	16.40	0.76	15.64	16.31	1.80	14.51
Magnesium	2.42	0.21	2.21	2.78	0.86	1.92
Potassium	0.78	0.14	0.64	0.71	0.25	0.46
Total	19.60	1.11	18.49	19.80	2.91	16.89
Soil 4						
Calcium	22.44	1.78	20.66	22.76	3.48	19.28
Magnesium	1.53	0.32	1.21	1.52	0.54	0.98
Potassium	0.63	0.21	0.42	0.61	0.30	0.31
Sodium	2.11	0.90	1.21	2.12	0.97	1.15
Total	26.71	3.21	23.50	27.01	5.29	21.72

EFFECT OF ALTERNATE WASHING AND DRYING ON THE pH OF SOILS

It was discovered while experimenting with soil 5 that, although boiling up the dried soil with water and evaporating off the water had no effect on the pH

TABLE 3

Effect of alternate washing and drying on pH of soils

	SOIL 2	SOIL 2A*	SOIL 5	SOIL 6	SOIL 7	SOIL 8	SOIL 9	SOIL 10	SOIL 11	SOIL 12	SOIL 13
pH of original soil	6.88	4.47	4.37	5.19	6.60	6.88	5.78	5.00	5.83	4.94	4.05
pH after 48 hours in oven	6.52	3.89	3.41	4.62	6.27	6.39	5.42	4.31	5.26	4.22	3.58
pH after washing and reheating for 48 hours	6.10	3.47	3.22	4.43	6.10	6.56	5.70	4.15	4.51	4.11	3.57
pH after washing and reheating for 48 hours	5.76	3.64	2.99	4.26	5.99	6.11	5.31	4.21	4.03	3.64	3.56
pH after washing and reheating for 48 hours	5.27	3.24	2.99	4.11	5.87	6.35	5.44	3.99	3.62	3.25	....
pH after washing and reheating for 48 hours	5.06	3.00	2.99	4.05	5.67	5.69	5.40	3.63	3.86	3.11	....
pH after washing and reheating for 48 hours	4.85	3.39	....	3.99	5.58	6.07	5.08	3.68	3.70	3.10	....
pH after washing and reheating for 48 hours	4.51	3.00	....	3.92	5.35	5.66	4.89	3.42	2.89	2.77	....
pH after washing and reheating for 48 hours	4.27	3.69	....	3.84	4.56	5.53	4.98	3.28	2.80	2.54	....
pH after washing and reheating for 48 hours	4.04	3.64	....	3.64	5.05	5.80	4.96	3.18	2.73	2.65	....
pH after washing and reheating for 48 hours	3.95	2.98	....	3.62	5.06	5.50	4.88	3.40	2.97	2.81	....
pH after washing and reheating for 48 hours	3.80	3.34	....	3.54	4.16	5.71	4.33	3.25	3.09	2.45	....
pH after washing and reheating for 48 hours	3.68	3.05	....	3.42	4.89	5.12	4.46	3.28	3.39	2.51	....
pH after washing and reheating for 48 hours	3.40	....	....	3.38	4.85	....	4.00	3.20	2.95	2.36	....
pH after washing and reheating for 48 hours	3.40	....	....	3.32	4.75	4.24	3.94	3.22	2.90	2.37	....
pH after washing and reheating for 48 hours	3.40	....	....	3.22	4.64	3.65	3.84	3.20	3.20	2.76	....
pH after washing and reheating for 48 hours	3.41	....	....	3.10	4.48	3.99	3.75	3.13	3.02	2.35	....
pH after washing and reheating for 48 hours	....	....	....	3.03	4.39	3.10	3.61	3.13	2.88	2.76	....
pH after washing and reheating for 48 hours	....	....	....	2.92	4.31	3.27	3.43	3.01	2.84	2.36	....
pH after washing and reheating for 48 hours	....	....	....	2.92	4.28	3.05	3.20	3.01	....	2.26	....
pH after washing and reheating for 48 hours	....	....	....	....	4.14	3.08	3.36	....	....	2.16	....
pH after washing and reheating for 48 hours	....	....	....	....	3.96	2.61	3.29	....	....	2.23	....
pH after washing and reheating for 48 hours	....	....	....	....	3.78	2.40	3.12	....	....	....	....
pH after washing and reheating for 48 hours	....	....	....	....	3.72	2.50	3.10	....	....	....	....

TABLE 3—*Concluded*

	SOIL 2	SOIL 2A*	SOIL 5	SOIL 6	SOIL 7	SOIL 8	SOIL 9	SOIL 10	SOIL 11	SOIL 12	SOIL 13
pH after washing and reheating for 48 hours	....	....	....	....	3.59	2.62	3.15	....	....	....	....
pH after washing and reheating for 48 hours	....	....	....	....	3.36	....	2.95	....	....	....	....
pH after washing and reheating for 48 hours	....	....	....	....	3.38	....	3.11	....	....	....	....
pH after washing and reheating for 48 hours	....	....	....	....	3.26	....	2.72	....	....	....	....
pH after washing and reheating for 48 hours	....	....	....	....	3.04	....	....	....	....	....	....
pH of heated base-free soils	2.84	....	2.99	3.00	2.83	2.58	2.58	2.81	2.50	1.81	2.56

* Soil 2, after treatment with hydrogen peroxide.

of the soil, leaching the dried soil with distilled water and again heating, caused the pH to fall. On repeating the procedure several times the pH became constant at a figure closely approximating the pH of the base-free soil prepared by leaching with dilute hydrochloric acid, and in addition the soil was found to contain no exchangeable bases.

Further experiment on soil 2 demonstrated the fact that merely leaching the heated soil with water caused the pH to rise slightly as a result of partial recovery of the soil, but that on heating this leached soil for 48 hours the pH fell again to a figure below that of the soil before the leaching was commenced.

Again, the effect of boiling up the soil with water before leaching was investigated, and this showed that such treatment did not produce a bigger decrease in pH than the leaching with cold water alone. As a result of these experiments it was decided to investigate the effect of alternate leaching and drying on a number of different soils.

About 200 gm. of the soil was used at the start of each experiment, and each soil was first heated for 48 hours in the steam oven at approximately 98°C. The pH was measured and then the soil was leached with about 2,000 cc. of water. The washing was done by decantation in a beaker, all the liquid being run through a filter so as to avoid any loss of fine material. At the conclusion of the leaching the soil was washed off the paper and beaker with hot water into an evaporating dish, and the liquid was evaporated off on the water bath. The soil was then passed through a 1-mm. sieve and heated for 48 hours in the oven again. The whole process was then repeated a number of times and the results are given in table 3. In the case of soil 7 the experiment had to be stopped before the end of the reaction because all the soil had been used up.

The results showed that in all cases except soils 2, 12, and 13, the pH tended to become constant at a value approximating that of the base-free soil prepared by leaching with dilute hydrochloric acid.

It is interesting to note that the reaction of the peat soil 13, although becoming more acid after the original heating in the oven, remained constant following the subsequent washing and drying operations. This indicates that the changes occurring in the other soils are a function of the inorganic constituents of the soils.

TABLE 4

Effect of alternate washing and drying on the water-soluble constituents of soil

Results in mgm. per 100 gm. dry soil

*Soil 6**

	CALCIUM	ALUMINUM	SULFATE
A	40.46	1.88	103.60
B	28.55	0.88	70.56
C	20.22	2.17	60.65
D	17.30	3.38	55.70
E	15.06	2.26	38.98
F	11.98	3.60	42.70
G	6.29	2.54	37.13
H	5.24	3.32	30.94
I	8.24	3.61	37.13
J	5.99	3.39	47.02
K	5.99	3.60	63.10
L	2.99	4.03	69.95
M	2.55	3.60	30.94
N	1.50	3.39	37.13
O	1.05	3.39	34.03
P	1.50	3.80	41.46
Q	3.75	4.23	54.45
R	2.25	3.68	55.70
S	1.20	4.45	32.19
T	4.49	3.84	33.42
U	3.75	3.68	44.55
V	4.49	3.93	34.03
W	3.75	3.60	35.28
X	1.50	3.45	25.99
Y	1.50	3.84	23.52
Z	1.05	3.60	21.05
a	0.72	3.76	43.93
b	0.72	3.53	37.13
c	0.00	3.38	29.70
d	0.00	3.45	33.42
e	0.00	4.51	41.51
f	0.00	4.51	26.18
Total..................	204.08	110.28	1,373.07
Exchangeable calcium left in soil...............	0.00	......	....
Total exchangeable calcium................	202.30	......	....

TABLE 4—*Continued*

Soil 14†

	CALCIUM	IRON	ALUMINUM	SULFATE	PHOSPHATE
A	80.40	0.5	2.25	394.4	Trace
B	57.00	1.22	2.0	340.0	5.04
C	52.60	2.92	2.25	252.0	5.60
D	46.00	2.05	3.0	236.0	5.80
E	42.40	4.43	3.0	148.0	5.71
F	34.40	4.56	3.0	228.0	5.09
G	28.60	3.76	3.0	132.0	4.08
H	24.00	7.49	4.0	148.0	3.90
I	38.00	8.77	7.0	148.0	3.09
J	20.00	11.30	9.0	152.8	2.50
K	16.0	11.43	10.0	194.4	2.00
L	18.0	11.43	10.0	84.0	1.60
M	20.93	8.25	10.0	110.4	1.16
N	11.6	11.80	12.0	132.0	1.26
O	12.4	9.00	8.0	95.2	0.72
P	4.4	7.00	8.0	60.0	0.32
Q	2.67	8.75	8.0	68.0	0.64
R	2.8	8.12	7.0	76.0	0.47
S	3.73	8.32	8.0	64.0	0.40
T	2.67	2.88	3.2	30.4	0.59
U	2.4	4.00	4.0	32.0	1.12
V	2.4	3.75	4.0	27.4	1.12
W	2.0	3.62	3.5	25.6	0.93
X	2.4	5.00	4.0	36.0	0.55
Y	2.27	3.50	3.0	21.6	0.48
Z	2.0	3.65	2.1	21.6	0.79
a	2.4	3.44	3.0	30.4	0.50
b	1.6	4.00	3.0	22.66	0.70
c	1.33	2.78	3.0	17.2	0.30
d	2.0	3.50	3.0	25.2	0.46
e	0.8	2.00	3.0	44.0	0.51
f	0.8	1.11	2.0	33.2	0.31
g	1.04	1.58	3.0	22.8	0.45
h	0.8	2.37	1.0	6.8	0.16
i	0.8	1.33	1.0	16.0	0.13
j	0.8	1.77	2.0	26.0	0.39
k	1.2	1.69	1.0	10.0	0.17
l	1.04	1.55	1.0	14.8	0.14
m	0.8	1.82	1.0	6.0	0.20
n	0.8	1.45	2.0	18.0	0.08
o	0.8	1.06	1.0	10.0	0.04
p	0.8	1.80	1.0	15.2	0.00
q	0.64	1.04	1.0	16.0	0.04
r	0.64	0.44	1.0	10.0	0.05
s	0.00	0.80	1.0	8.0	0.05
t	0.00	0.66	1.0	7.2	0.05
u	0.00	0.99	0.5	6.8	0.10
Total..................	551.16	194.68	178.8	3,624.06	59.79
Exchangeable calcium remaining in soil......	0.00	......	.....	....	.....
Total exchangeable calcium................	560.00	....	......	....	....

TABLE 4—*Concluded*

Soil 15‡

	CALCIUM	IRON	ALUMINUM	SULFATE	CHLORIDE
A	12.12	0.86	3.33	21.41	4.23
B	4.0	0.8	1.0	13.2	3.50
C	3.2	0.37	0.25	11.2	Nil
D	6.67	1.0	2.0	31.2	Nil
E	4.0	1.33	2.0	23.2	
F	4.0	1.5	1.0	23.2	
G	1.33	0.99	1.0	16.4	
H	2.96	0.67	1.0	10.0	
I	1.2	0.90	1.0	9.2	
J	0.8	1.66	0.5	5.2	
K	0.56	0.58	1.0	11.2	
L	2.0	0.8	0.25	5.2	
M	0.8	0.62	1.0	9.2	
N	0.0	0.8	0.5	4.2	
O	0.8	1.03	1.0	6.0	
P	0.8	1.12	1.0	6.8	
Q	1.2	0.32	1.0	10.8	
R	0.0	0.33	0.5	4.0	
S	0.0	0.28	0.45	2.8	
T	0.0	0.30	0.4	3.2	
Total..................	46.44	16.26	20.18	227.61	7.73
Exchangeable calcium left in soil............	182.60	.....	.....	....	....
Total exchangeable calcium................	236.80	.....	......	....	....

Soil 16§

	CALCIUM	IRON	ALUMINUM	SULFATE	PHOSPHATE	CHLORIDE
A	2.93	0.92	3.0	30.0	Trace	0.70
B	2.67	0.70	3.0	26.0	Nil	Nil
C	2.00	0.70	3.0	27.2	Nil	Nil
D	1.33	0.88	1.0	24.0	Trace	
E	1.33	1.50	1.0	14.0	Nil	
F	1.20	0.77	1.0	11.2	Nil	
G	1.20	0.64	1.0	6.8	Nil	
H	1.20	0.71	1.0	8.0		
I	0.56	1.06	1.0	9.2		
J	0.00	0.58	1.0	7.6		
K	0.00	0.71	0.5	5.2		
L	0.00	0.75	1.0	7.6		
Total...........	14.42	9.92	17.5	176.8	.....	0.7
Exchangeable calcium left in soil..........	0.00	....	....	.....	.....	....
Total exchangeable calcium...	12.60	....	....	.....	......	....

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* Soil 6 A nitrite present, chloride nil; 6 B nitrite faint trace, chloride nil; 6 C nitrite very faint trace, chloride nil; 6 D chloride nil.

† Soil 14 A nitrate trace; chloride nil; 14 B nitrate and chloride nil; 14 C nitrate nil.

‡ Soils 15 A and B phosphate and nitrate nil, 15 C phosphate nil.

§ Soils 16 A and B nitrate nil.

The object of the next set of experiments was to discover the anions in combination with which the bases came into the water solution during the process of alternate heating and washing the soils. Preliminary qualitative experiments showed that considerable quantities of sulfates were present in the aqueous extracts. As a result of this it was decided to make quantitative estimations of sulfates in the solution and also of other anions such as chloride, nitrate, phosphate. The following procedure was adopted: Twenty-five grams of soil which had been heated for 48 hours at 98°C. in the water oven were taken. The soil was placed in a beaker with 100 cc. of distilled water and left overnight with occasional stirring. The liquid was then decanted through a hard filter paper into a liter flask, after which the soil was all washed on to the filter. The soil was then leached with distilled water until the filtrate was exactly 1 liter. Estimations of the calcium, sulfate, etc., in this solution were made and the results are given as A in table 4. The soil was then heated for 48 hours in the oven without removing it from the filter paper, after which a liter of water was leached through it. The results of the analysis of this extract are given as B in table 4. The procedure was then repeated a number of times until all the calcium had been removed, the results being given as C, D, E, F, etc., in table 4.

The calcium was determined by precipitation as oxalate and titration with potassium permanganate, the iron by a colorimetric method with ammonium thiocyanate, the aluminum by the colorimetric method of F. W. Atack with alizarin S (1), and the phosphate by the colorimetric method of F. W. Parker and J. F. Fudge (5).

The sulfate was determined by weighing as barium sulfate in the case of soil 6, and in the other soils by the colorimetric method of Gedroiz (4). In addition the amount of exchangeable calcium was determined in the original soil and also the amount of exchangeable calcium remaining after the leaching and drying operations.

The results show that with the exception of soil 15 the total calcium in the water extracts is equal to the amount of exchangeable calcium originally present in the soil, and that, soil 15 again excepted, the soils at the end of the treatment contain no exchangeable calcium. This indicates that in certain soils it is possible to remove all the exchangeable calcium, and doubtless all the other exchangeable bases, by this process of alternate heating and leaching. Further, the results indicate that the exchangeable bases come into solution almost entirely as sulfates.

It seems in addition that the iron and aluminum also come into solution as sulfates. In the case of soils 6 and 14 a large quantity of sulfate was available to come into solution and in both these soils all the exchangeable calcium was removed by the washing and drying operations. In the case of soils 15 and 16 comparatively little sulfate could come into solution. Soil 16 contained very little exchangeable calcium and there was enough sulfate available to cause the removal of all this calcium. Soil 15, however, contained considerably more exchangeable calcium and there was not enough sulfate coming into solution to bring out all of it, hence the removal of calcium was incomplete.

It is noteworthy that the iron and aluminum sulfates continue to come into solution after all the exchangeable calcium has been removed. A qualitative test proved that the soils still contained sulfates even after the prolonged leaching and drying operations. Possibly the sulfate is absorbed in the soil complex as sulfates of iron and aluminum, and the drying of the soil releases some of these salts which react with the exchangeable bases of the soil.

The experimental work has demonstrated the following facts:

1. The amounts of bases extractable by ammonium (or sodium) chloride are the same before and after heating the soil.
2. There is an increase in water-soluble bases after heating the soil.
3. Alternate washing and heating the soils cause the pH to fall to a figure approximating that of the base-free soils, except in the case of the peat and the soil from Dodoma, Tanganyika.
4. It is possible in some soils to remove all the exchangeable bases by a process of alternate washing and drying at 98°C.
5. The bases go into solution largely as sulfates.
6. The iron and aluminum also go into solution as sulfates and continue to come into solution after all the calcium has been removed.
7. The amount of bases removed from the soil in the water extract after heating depends on the quantity of SO_4'' which can come into solution.
8. The soils still contain sulfates at the end of the washing and heating operations.

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STUDIES ON THE BLUE COLORIMETRIC METHOD FOR THE DETERMINATION OF PHOSPHORUS[1]

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A review of the various blue colorimetric methods proposed for the determination of phosphorus indicated that no one method[2] had been critically tested with reference to the possible effect of such concentrations of salts as are found in many soils and irrigation waters of semiarid regions. In a study of this question certain additional information was obtained. It is the purpose of this paper to present briefly the results of this work.

EXPERIMENTAL

The method employed in this investigation is that outlined by Truog and Meyer (2). As ordinarily used, 4 cc. of a reagent containing 2.5 per cent ammonium molybdate in 10 *N* sulfuric acid is added to the solution to be tested, the volume increased to 99.5 cc., and then while the volume is being shaken, 6 drops of a 2.5 per cent solution of a stannous chloride added. The blue color developed if phosphate or arsenate be present is then compared with similarly prepared standards. Under certain conditions it is desirable to keep the ammonium molybdate and acid as separate reagents, but in any case the final proportions used should be as indicated in the foregoing.

Effect of varying amounts of stannous chloride

In connection with the use of permanent standards (1), attention has been directed to the fact that stannous chloride solutions supposedly protected from the air by mineral oil will, nevertheless, gradually oxidize. This oxidation is caused by the diffusion of the oxygen of the air through the oil layer. To obtain quantitative information on the effect of small and large variations of stannous chloride, a series of solutions containing the same quantities of PO_4 was prepared, different amounts of this reagent were added to each solution, and at frequent intervals during the period of one hour, comparisons of the color intensity developed were made with permanent glass color standards.[3] To compensate for the gradual appearance of a greenish brown tint, which is caused by the action of stannous chloride upon the ammonium molybdate itself, solutions containing all the reagents minus phosphate were prepared and placed in the control compartment of the Hellige Comparator. Thus the readings represent only that color produced by the ammonium phosphomolybdate itself. The data obtained are recorded in table 1. The relation between the amount of stannous chloride added and the maximum color attained is shown more clearly in figure 1. Up to 0.60 mgm. $SnCl_2 \cdot 2H_2O$ the color is proportional to the amount of reducing agent added. Larger quantities create definite but relatively small increases in color. The addition of 7.38 mgm. $SnCl_2 \cdot 2H_2O$ corresponds to the quantity used by Truog and Meyer (2). It is evident that fairly large deviations from this amount will effect but minor variations in color.

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[2] Toward the close of this investigation a method was published by Zinzadze (4) in which the necessity for using a reducing agent is eliminated. He has reported the effect of varying quantities and kinds of salts on the determination.

TABLE 1

Effect of varying amounts of $SnCl_2 \cdot 2H_2O$ on total color produced from a given PO_4 concentration

MILLIGRAM OF $SnCl \cdot 2H_2O$ ADDED	READING OF COLOR DISC AT SUCCEEDING INTERVALS FOLLOWING $SnCl_2$ $2H_2O$ ADDITION																
	Minutes																
	1	2.5	5.0	7.5	10.0	12.5	15.0	17.5	20.0	22.5	25.0	27.5	30.0	35.0	40.0	45.0	60.0
0 24	0.7	0.7	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0 5	0.5	0.5	0.2
0 37	1.7	1.7	1.5	1.4	1.4	1.4	1.4	1.4	1.4	1.3	1.4	1.3	1.3	1.3	1.3	1.3	1.3
0.48	2.5	2.7	2.7	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
0.60	3.2	3.3	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2
0 72	3.5	3.5	3.5	3.4	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2
1.23	3.7	3.8	3.8	3.8	3.4	3.3	3.3	3.4	3.3	3.3	3.4	3.4	3.4	3.4	3.4	3.4	3.4
2.46	3.8	3.8	3.8	3.8	3.8	3.5	3.4	3.3	3.3	3.3	3.3	3.3	3.3	3.4	3.3	3.4	3.4
3.69	3.9	4.0	4.2	4.2	4.2	3.9	3.7	3.4	3.3	3.3	...	3.4	3.3	...	...	...	...
7.38*	4.0	4.2	4.2	4.3	4.2	4.1	4.1	4.1	3.7	3.3	3.3	3.3	3.4	3.2	3.3	3.3	3.3
12.3	3.8	4.2	4.3	4.4	4.4	4.4	4.3	4.3	4.3	4.3	4.3	4.0	3.8	3.7	3.7	3.4	3.4
14.7	3.8	4.2	4.3	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.4	4.4	4.0	3.7	3.4	3.2	3.2

* Corresponds to amount of $SnCl_2 \cdot 2H_2O$ used in Truog and Meyer method.

In addition to the foregoing information these data permit some speculation regarding the chemistry involved in this reaction. It will be noted that amounts in excess of 0.60 mgm. $SnCl_2 \cdot 2H_2O$ give a maximum color corresponding to a reading of more than 3.2; however, upon standing, the color fades back to this same level and then remains apparently constant for a long period. This suggests that two or more blue colored compounds are formed, one easily reduced and relatively stable, the other more difficulty reduced and stable only in the presence of excess reducing agent. Wu (3) has presented evidence which indicates that phospho-molybdate complexes can be reduced to different oxidation products. The aforementioned interpretation is thus in harmony with his data.

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[3] These color standards, ten in number, are mounted on a circular frame especially designed for use in the Hellige Comparator. Each standard is designated by an Arabic numeral and varies sufficiently in color intensity from those adjacent to permit interpolated readings.

Effect of SiO_2

According to Truog and Meyer (2), SiO_2 may be present in the final solution in concentrations up to 700.0 p.p.m. without interfering with the usual phospho-molybdate color. It has been found, however, that this noninterference depends upon the manner in which the acid and molybdate reagent are combined with the solution being tested. In solutions of low acidity SiO_2 will unite with molybdate to form a yellow silico-molybdate. This compound will then persist even after the acidity is increased to that concentration requisite for the phospho-molybdate reduction. Upon the addition of stannous chloride this silico-molybdate compound will be reduced to a blue color. After a careful study of various methods of combining reagents it was found that conditions are most favorable for the formation of the aforementioned compound when the molybdate reagent is mixed first with the unknown followed then by the slow drop by drop addition of acid. Under these circumstances as little as 0.5 p.p.m. SiO_2 may produce a blue color. On the other hand, if the acid is first added and then the molybdate drop by drop, as much as 700 p.p.m. SiO_2 may be present without interfering.

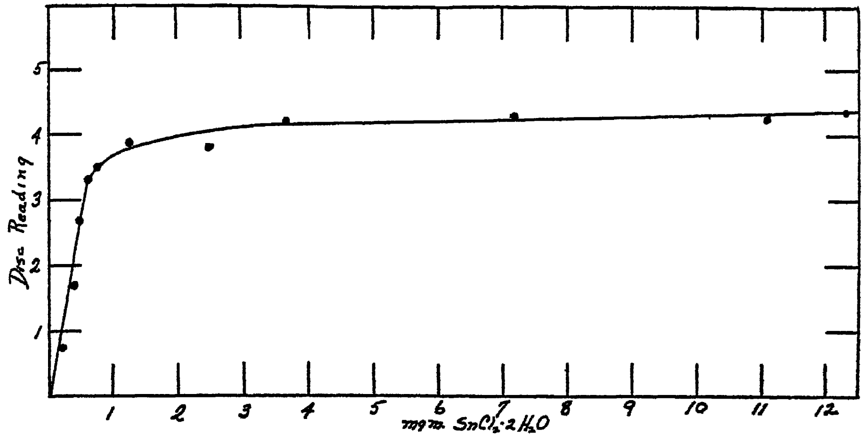

FIG. 1. EFFECT OF VARYING AMOUNTS OF $SnCl_2 \cdot 2H_2O$ ON TOTAL COLOR PRODUCED

If the molybdate and acid are united as one reagent the chances of interference by SiO_2 are greatly diminished but still not entirely eliminated. For example, if the first cubic centimeter of acid-molybdate reagent is added drop by drop to a slightly alkaline or neutral solution containing SiO_2, some silico-molybdate will be formed. If, however, this procedure is reversed by adding the solution *to* the acid-molybdate reagent, conditions of acidity are such as to prevent the formation of this compound.

With phosphate or arsenate the manner of combining the acid and molybdate with the solution has no apparent influence upon the formation of their respective molybdates. The determination of SiO_2 in the presence of phosphate or arsenate is thus made possible.

Influence of iron

The author has confirmed the results of Truog and Meyer (2) in which they found that *ferric* iron, in amounts exceeding 4–6 p.p.m. interferes with the determination. This effect can be eliminated by reducing the ferric iron.

TABLE 2

Effect of ferrous iron upon phosphorus determination

CONCENTRATION OF FERROUS IRON IN FINAL SOLUTION	INTERVAL BETWEEN MAKING TO FINAL VOLUME AND ADDITION OF $SnCl_2 \cdot 2H_2O$	CONCENTRATION PO_4 IN STANDARD	CONCENTRATION PO_4 FOUND
p.p.m.	*minutes*	*p.p.m.*	*p.p.m.*
10.0	None	0.76	0.74
10.0	None	0.76	0.75
10.0	None	0.76	0.75
10.0	1.0	0.76	0.66
10.0	3.0	0.76	0.54
10.0	5.0	0.76	0.50
10.0	15.0	0.76	0.48
10.0	90.0	0.76	0.47
10.0	960.0	0.76	0.48

TABLE 3

Influence of varying concentrations of ferrous iron on diminution of blue color

CONCENTRATION OF FERROUS IRON IN FINAL SOLUTION	INTERVAL BETWEEN MAKING TO FINAL VOLUME AND ADDITION OF $SnCl_2$ $2H_2O$	CONCENTRATION PO_4 IN STANDARD	CONCENTRATION PO_4 FOUND
p.p.m.	*minutes*	*p.p.m.*	*p.p.m*
None	5.0	0.76	0.75
1.0	5.0	0.76	0.62
10.0	5.0	0.76	0.50
50.0	5.0	0.76	0.42

However, it has been found that under certain conditions *ferrous* iron may also affect the results. If the test solution containing phosphate and ferrous iron is allowed to stand after being brought to final volume (99.5 cc.) *before* the addition of stannous chloride, the iron will diminish the color that would otherwise have been produced.

To determine how long a solution may thus stand before this effect begins, the following experiment was made: Solutions containing phosphate and 10 p.p.m. Fe as ferrous ammonium sulfate were brought to a volume of 99.5 cc. and allowed to stand for 1, 3, 5, 15, 90, and 960 minutes respectively before the reducing agent was added. The color developed was then compared with similarly prepared solutions containing no iron, as shown in table 2. The results indicate that ferrous iron in this concentration exerts an effect if the solution stand[illegible]s only 1 minute, and has its maximum effect in about 5 minutes.

The effect of varying quantities of ferrous iron are shown in table 3. Solutions were prepared containing equal amounts of PO_4 but different amounts of ferrous ammonium sulfate. After being made to volume, they were allowed to stand 5 minutes, the stannous chloride was added, and the color was compared with that of iron-free solutions. It was found that under these conditions as little as 1 p.p.m. of ferrous iron will introduce an error. However, if the stannous chloride is immediately added, as high as 750 p.p.m. ferrous iron produced no effect. Under conditions of acidity higher than that of the final solution (0.4 *N*), ferrous iron apparently does not interfere. Hence it is better practice to add the unknown *to* the acid or acid-molybdate reagents. By so doing the acidity of the solution during the process of mixing is always higher than that of the final solution. By following this procedure and adding the stannous chloride as soon as final volume has been attained, no trouble from ferrous iron will be enountered.

Effect of nitrates, chlorides, and sulfates

In studying the influence of nitrates, chlorides, and sulfates upon the determination of phosphate, attention was given both to the immediate and the continued effects. The immediate influence of these salts was determined in solutions of constant PO_4 content but variable amounts of these salts. The color comparisons between standard and unknown were made 2–3 minutes after the addition of the stannous chloride. The continued effect was studied by making comparisons with permanent standards after the lapse of varying periods. The results are given in tables 4 to 9.

Table 4 shows the immediate effect of different nitrates. Above 140 p.p.m. of N, HNO_3 brings about a gradual diminution of the blue phosphate. On the other hand, KNO_3, $NaNO_3$, and NH_4NO_3, in amounts as high as 1,400.0 p.p.m. N, have no immediate influence on the test. Greater amounts slightly intensify the blue color, but even with as much as 12,040 p.p.m. N present in the form of these nitrates, there is less than a 10 per cent increase in the color intensity. In contrast to the effect of these compounds calcium nitrate produces no important effect other than that $CaSO_4$ may be precipitated. This, of course, makes precise readings difficult.

The influence of varying concentrations of $NaNO_3$ on the reaction (table 5), when observed over a period of 1 hour, demonstrates the effect of nitrates upon the fading reaction. With solutions containing no nitrate, the color intensity reaches a maximum corresponding to a reading on the color disc of 4.0. It

TABLE 4

Immediate effect of nitric acid and nitrates upon phosphorus determination

COMPOUND USED	CONCENTRATION OF PO_4 FOUND IN SOLUTIONS WITH VARYING NO_3 CONCENTRATION							
	P.p.m. N as NO_3 in final solution							
	None	1.4	14.0	140.0	700.0	1,400.0	7,000.0	12,040.0
HNO_3	0.75	....	....	0.73	0.69	0.62	....	....
KNO_3	0.75	0.74	0.75	0.74	0.74	0.78	0.83	0.82
$NaNO_3$	0.75	....	0.75	0.75	0.77	0.76	....	0.80
$Ca(NO_3)_2$	0.74	0.78	0.76	0.76	....	0.76	0.73	0.76*
NH_4NO_3	0.75	0.76	0.75	0.78	0.77	0.77	0.82	0.82

*A precipitate of $CaSO_4$ came down at this concentration; hence value is only approximate.

TABLE 5

Effect of $NaNO_3$ on reaction over a period of 60 minutes

CONCENTRATION OF N AS $NaNO_3$ IN FINAL SOLUTION	READING OF COLOR DISC AT SUCCEEDING INTERVALS FOLLOWING $SnCl_2 \cdot 2H_2O$ ADDITION																
	Minutes																
	1	2.5	5.0	7.5	10.0	12.5	15.0	17.5	20.0	22.5	25.0	27.5	30.0	35.0	40.0	45.0	60.0
p.p.m.																	
None	3.7	3.9	3.9	3.9	4.0	4.0	4.0	4.0	4.0	4.0	3.7	3.4	3.2	3.2	3.2	3.2	3.2
1.4	3.5	3.8	3.9	3.9	3.9	3.9	3.9	3.9	3.6	3.4	3.3	3.2	3.2	3.0	3.0	3.0	3.0
14.0	3.5	3.7	3.9	3.9	3.9	3.9	3.9	3.9	3.9	3.6	3.3	3.1	3.0	3.0	3.0	3.0	3.0
140.0	3.7	3.9	3.9	3.9	3.9	3.9	3.9	3.9	3.8	3.3	3.2	3.0	2.9	2.9	2.9	2.9	2.9
700.0	3.5	3.9	3.9	3.9	3.9	3.9	3.9	3.9	3.8	3.5	2.8	2.1	1.9	1.2	0.8	0.5	0.2
1,400.0	3.7	4.0	4.0	4.0	4.0	4.0	4.0	3.7	2.8	2.2	1.7	1.4	1.0	0.5	*	*	*
7,000.0	3.7	4.0	4.0	4.0	4.0	4.0	2.8	2.7	1.9	...	1.0	0.7	0.5	0.1	*	*	†
12,040.0	3.9	3.9	4.0	4.0	4.0	3.9	3.7	1.9	0.9	0.7	0.5	...	...	...	†	...	...

* Slight color.

† All gone.

TABLE 6

Effect of sulfates upon phosphorus determination

SALT USED	CONCENTRATION OF PO_4 FOUND IN SOLUTIONS WITH VARYING SO_4 CONCENTRATION							
	P.p.m. SO_4 in final solution							
	None	4.8	48.0	480.0	2,400,0	4,803.0	24,015.0	41,300.0
K_2SO_4	0.76	0.76	0.75	0.73	0.78	0.79	0.87	0.87
$(NH_4)_2SO_4$	0.76	0.78	0.78	0.77	0.82	0.88	0.92	0.87
$MgSO_4$	0.78	0.78	0.79	0.76	0.78	0.81	0.83	0.86

remains apparently constant at this value for about 22 minutes, after which it fades to a color level corresponding to a disc reading of 3.2. In solutions containing 140 p.p.m. N or more, fading begins sooner and proceeds to a greater degree than in nitrate-free solutions, both of these effects being more pronounced with higher nitrate concentrations. However, under the conditions of this method and at a temperature of 24°C. no measurable influence upon the reaction was detected within the first 10 minutes even when very high concentrations of the nitrate were present.

TABLE 7

Effect of K_2SO_4 on reaction over period of 60 minutes

CONCENTRATION OF SO_4 IN FINAL SOLUTION	READING OF COLOR DISC AT SUCCEEDING INTERVALS FOLLOWING $SnCl_2 \cdot 2H_2O$ ADDITION																
	Minutes																
	1	2.5	5.0	7.5	10.0	12.5	15.0	17.5	20.0	22.5	25.0	27.5	30.0	35.0	40.0	45.0	60.0
p.p.m.																	
None	3.7	3.9	3.9	3.9	4.0	4.0	4.0	4.0	4.0	4.0	3.7	3.4	3.2	3.2	3.2	3.2	3.2
480.3	3.8	3.9	4.0	4.0	4.1	4.1	3.7	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.1
4,803.0	4.1	4.2	4.4	4.4	4.3	4.4	4.4	4.1	3.6	3.6	3.6	3.4	3.4	3.3	3.3	3.2	3.2
24,015.0	4.7	5.0	5.1	5.2	5.3	5.3	4.8	4.5	4.5	4.4	4.3	4.3	4.2	4.2	4.2	4.1	4.0
41,300.0	4.8	5.0	5.2	5.3	5.4	5.4	5.4	5.4	5.4	4.3	4.3	4.3	4.2	4.2	4.1	4.0	3.9

TABLE 8

Effect of chlorides upon phosphorus determination

COMPOUND USED	CONCENTRATION OF PO_4 FOUND IN SOLUTIONS WITH VARYING Cl CONCENTRATIONS							
	P.p.m. Cl in final solution							
	None	3.5	35.4	354.6	1,773.0	3,546.0	17,730.0	30,500.0
HCl	0.76	....	0.77	0.76	0.65	0.58	....	....
NaCl	0.76	0.79	0.78	0.75	0.73	0.71	0.59	0.53
KCl	0.76	0.79	0.79	0.77	0.78	0.72	0.58	0.52
NH_4Cl	0.76	0.79	0.77	0.77	0.73	0.71	0.59	0.52

TABLE 9

Effect of NH_4Cl on reaction over period of 60 minutes

CONCENTRATION OF Cl IN FINAL SOLUTION	READING OF COLOR DISC AT SUCCEEDING INTERVALS FOLLOWING $SnCl_2 \cdot 2H_2O$ ADDITION																
	Minutes																
	1	2.5	5.0	7.5	10.0	12.5	15.0	17.5	20.0	22.5	25.0	27.5	30.0	35.0	40.0	45.0	60.0
None	3.7	3.9	3.9	3.9	4.0	4.0	4.0	4.0	4.0	4.0	3.7	3.4	3.2	3.2	3.2	3.2	3.2
354.6	3.7	4.0	4.0	4.0	4.0	4.0	4.0	4.0	3.5	3.2	3.2	3.2	3.2	3.2	3.2	3.2	3.2
3,546.0	3.5	3.7	3.8	3.8	3.7	3.7	3.5	3.0	2.9	2.8	2.8	2.7	2.7	2.7	2.7	2.7	2.7
7,730.0	2.9	2.9	2.9	2.9	2.7*	2.5*	2.5*	2.4*	2.3*	2.2*	2.2*	2.2*	2.2*	2.2*	2.2*	2.2*	2.0*
3,500.0	2.4	2.6	2.6	2.0*	2.0*	2.0*	1.8*	1.8*	1.8*	1.5*	1.5*	1.5*	1.5*	1.5*	1.5*	1.5*	1.5*

* Color tint in solution caused by high chlorides makes close readings difficult.

Table 6 shows the immediate effect of various sulfates. Solutions containing 2,400 p.p.m. SO_4 or more, somewhat intensify the phosphate color, but the effect is not proportional to the amount of sulfate. Solutions containing 41,300 p.p.m. SO_4, intensified the color less than 15 per cent. There appears to be no difference in the behavior of K_2SO_4, $(NH_4)_2SO_4$, and $MgSO_4$.

The influence of varying amounts of K_2SO_4 on the reaction over a period over a period of 1 hour is shown in table 7. Except for the increase in color which high sulfate produces, there appears to be no other important effect.

The immediate effect of varying concentrations of HCl, NaCl, KCl, and NH_4Cl is shown in table 8. Unlike nitrates and sulfates, high concentrations of chlorides diminish the color. With concentrations of 3,546.0 p.p.m. Cl in the final solution these salts decrease the color about 5 per cent. Corresponding, though not proportional, decreases occur with higher concentrations. The action of free HCl is probably due in part to its effect on the H-ion concentration and is similar to that of other chlorides except that the effect occurs with smaller quantities of the acid.

The effect of NH_4Cl on the reaction over a period of 60 minutes is shown in table 9. In addition to reducing the total color produced, this salt when present in amounts of 3,546.0 p.p.m. Cl or more, causes the fading reaction to begin sooner. However, the degree of fading is apparently no greater than that which occurs in salt-free solutions, which is in contrast to the effect of nitrates.

Summarizing briefly it may be said that the immediate effect of nitrates and sulfates is to increase the color slightly, whereas high chlorides materially decrease the color. Upon standing, nitrates in amounts over 140 p.p.m. cause fading of the blue phosphate color to begin sooner and proceed to a greater degree, the magnitude of this effect depending upon the amount of nitrate present. Other than the initial increase in color, sulfates appear to have no influence on the reaction. Chlorides produce a more immediate but less intense fading than nitrates.

Miscellaneous notes on test

Effect of vanadium. Solutions containing as high as 25 p.p.m. of vanadium as ammonium meta vandate produced no interfering color under the conditions of the determination. Other possible influences of this element have not yet been investigated.

Arsenic. The wide distribution of arsenic in nature, particularly its known presence in certain waters and soils of semiarid regions, its presence in certain phosphate fertilizers, and the possible contamination of plant tissue and soils where arsenicals have been applied as insecticides, emphasize the necessity for being on guard against contamination from this source. As pointed out by Truog and Meyer (2), arsenates are the only compounds of arsenic that interfere. When present, arsenates can be reduced to the trivalent state, which compounds do not interfere with the phosphate determination.

Acidity of final solution. The necessity for maintaining a constant ratio of acid to molybdate should again be emphasized. With a given molybdate concentration too little acid permits some of this constituent to be reduced, giving rise to a blue color, whereas too high an acidity prevents full color development from the ammonium phospho-molybdate present.

Concentration of PO_4 in standard and unknown. The PO_4 concentration of fresh standards should be as near as possible to that of the unknown being tested. The color developed is not strictly proportional to the amount of PO_4 present and serious errors may arise from failure to observe this precaution.

Organic phosphorus. Determination of phosphate in soil extracts colored with organic matter may be inaccurate partly because of the interfering color tints that are developed and the possible interference of such substances on the normal reaction. When such extracts are ignited with $Mg(NO_3)_2$, the determination will then represent both the inorganic and organic phosphorus originally present. However, it is questionable whether the difference between the intensity of the blue color produced by the original solution and that of the oxidized solution should be attributed entirely to organic phosphorus. Inaccuracies in the former determination and the possible presence of inorganic phosphorus in forms other than PO_4 may obviously lead to erroneous conclusions regarding the amounts of organic phosphorus present. Further study on this point is needed.

SUMMARY

Studies on the Truog and Meyer (2) blue colorimetric method for the determination of phosphate have been made. It has been found that stannous chloride solutions, although protected from the air by a layer of mineral oil, nevertheless gradually becomes oxidized. If the reagent is made up in 2-liter quantities, stored in bottles of small cross section, and protected by 1 cm. thick layers of mineral oil, the reagent may be used several months before its deterioration becomes serious.

Under conditions of low acidity, silica may form a silico-molybdate which persists even after the acidity is increased. Upon the addition of stannous chloride this compound undergoes reduction to a blue color indistinguishable from that of the phospho-molybdate. By combining the reagents in a manner favorable to the formation of the silico-molybdate as little as 0.5 p.p.m. SiO_2 may produce an error. On the other hand if the acid and molybdate are combined into one reagent and the test solution is always added to the required amount of this reagent, as much as 700 p.p.m. SiO_2 may be present without appreciable interference.

Ferrous iron in amounts as small as 1 p.p.m. in the final solution diminishes the total color producible if the test solution, after being made to volume (99.5 cc.), is allowed to stand before the addition of stannous chloride. If the reducing agent is added immediately, ferrous iron may be present in large amounts without interfering.

Only in the presence of comparatively high concentrations of nitrates, sulfates, or chlorides, is there any effect upon the accuracy of the test.

When used in accordance with the precautions noted in this paper the Truog and Meyer method combines simplicity, speed, and accuracy, and can be adapted to the determination of phosphorus under a wide range of conditions.

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ON THE ORIGIN OF THE URONIC ACIDS IN THE HUMUS OF SOIL, PEAT, AND COMPOSTS[1]

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The carbohydrates known as hemicelluloses comprise a number of different polysaccharides which give on hydrolysis with hot dilute mineral acids, at atmospheric pressure, not only sugars (hexoses or pentoses or both), but also uronic acids, frequently methoxyl groups and sometimes acetyl groups. Tollens (41) was the first to express the opinion that gums contain d-glucuronic acid. His conclusions were not based upon the isolation of the specific substance, but merely upon certain specific group reactions. Since then, uronic acids have been definitely isolated from a number of the so-called gums, mucilages, pectins, and similar complexes (8, 16, 33), and have been found to be universally distributed in the plant kingdom, as well as to occur also in animal tissues and in microbial cell substance.

The fact is becoming more and more universally recognized that very few hemicelluloses are pure polysaccharides or polymers of only one sugar; on the contrary, most of the preparations so far obtained contain more than one sugar, or mixtures of sugars and uronic acids (25, 18, 21, 24). Before a systematic classification of this group of carbohydrates, based upon the type of sugars and sugar acids produced on hydrolysis, has been developed, all hemicelluloses, gums, mucilages, pectins, and related compounds can be grouped together. This large heterogeneous group of carbohydrates can then be separated into two general subgroups, comprising, (*a*) those complexes that give only sugars, on hydrolysis with dilute mineral acids, or the true hemicelluloses, including hexosans and pentosans; and (*b*) the polyuronides, or those complexes that give, on hydrolysis with mineral acids, uronic acids, in addition to sugars and other compounds, as in the case of the pectins, various gums, etc. (4, 5, 19, 20).

The various methods of analysis of plant tissues for hemicelluloses are based either upon the determination of one group of complexes, such as the pentosans by the furfuraldehyde method, or the several groups, as by hydrolysis with hot dilute mineral acids, after the sugars and starches have been removed. Both of these procedures are far from giving an accurate quantitative determination of the actual content of hemicelluloses. In the first method, which is based upon hydrolysis of the pentosan and the subsequent transformation of the pentose to furfuraldehyde,

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$$(C_5H_8O_4)n + (n-1)H_2O = n(C_5H_{10}O_5)$$
$$C_5H_{10}O_5 = C_5H_4O_2 + 3\ H_2O$$

Some of the uronic acids will also be determined as pentosans, as shown by the following reaction:

$$C_6H_{10}O_7 = C_5H_{10}O_5 + CO_2$$

In the hydrolysis of hemicelluloses by dilute acids, some of the uronic acids will be destroyed. Heidelberger and Goebel (12) have shown, for example, that when aldobionic acid isolated from pneumococcus is hydrolyzed with 1*N* sulfuric acid for 20 hours, half of the molecule, namely the uronic acid, is largely destroyed. Butler and Cretcher (3) have also found that when aldobionic acid from gum arabic was hydrolyzed with 5 per cent sulfuric acid, d-galactose was obtained while the uronic acid was destroyed to a great extent. According to Weinmann (32), when gum arabic, with a uronic acid content of 17.6 per cent, is hydrolyzed with 2 per cent hydrochloric acid for one and a half hours, enough CO_2 is lost to be equivalent to 12 per cent of the total glucuronic acid of the gum. Link and Niemann (17), using purified uronic acids, have demonstrated that, on boiling with 1*N* sulfuric acid for 15 hours, the acid lost enough CO_2 to be equivalent to 55 per cent of the total glucuronic acid present; when lemon pectin, containing 88 per cent galacturonic acid, was hydrolyzed for 15 hours with 2 per cent sulfuric acid, an equivalent of 33.85 per cent of the acid was liberated as CO_2.

These results as well as others that could be cited (9, 11) are sufficient to illustrate the inaccuracies involved in the quantitative determination of hemicelluloses by the foregoing two procedures. In the method used by the senior author and his associates (37) for the proximate analysis of plant tissues and their decomposition products, such an error was constantly involved. This was fully recognized throughout all the determinations previously published. However, this error was not of such magnitude in the majority of plant substances employed, such as cereal straws, corn stalks, tree products, as to modify the determinations considerably. Since the method was only proximate and since the accounting of 90 to 95 per cent of the plant constituents was the maximum that one could expect under those conditions; it was felt that this method for hemicellulose determination was as good as any other one until a more accurate procedure could be developed; this was especially true because of the fact that the results were always comparable.

However, certain plant substances, such as sphagnum moss, and certain decomposed plant residues, such as peat, and one had a suspicion that it was also true of the humus or the organic matter of the soil itself, always gave a wide discrepancy which was due to the inaccurate determination of the hemicelluloses. In order to throw light upon this subject, the following investigations were undertaken.

HISTORICAL

With the advance of our knowledge of the complexity of the hemicelluloses, the information concerning the nature and mechanism of their decomposition and synthesis by microörganisms has been rapidly accumulating. It has been established that just as the hemicelluloses vary in their chemical structure, so they vary in the ability of microörganisms to attack them. The fact that a certain fungus or bacterium is capable of decomposing readily one type of hemicellulose is no proof that it will also attack others, frequently even closely related compounds. A marked specificity was found to exist between the chemical structure of these carbohydrates and the ability of microörganisms to attack them (34). This phenomenon is similar in certain respects to the early observations of Pasteur concerning the specificity of certain fungi in attacking the isomers of organic acids.

Some of the hemicelluloses are readily decomposed by a number of bacteria and fungi, as in the case of certain mannans and xylans, whereas others are more resistant to decomposition, especially the various galactans and some of the polyuronides. These phenomena have a direct bearing upon the processes of decomposition of plant and animal residues in soils and in composts and upon the chemical nature of the dark residual material, which is commonly known as humus. Although the plant residues which find their way into the soil are richer in cellulose than in hemicelluloses, the organic matter or humus of the soil itself, as well as of peat and composts, frequently contains no cellulose at all or very little of it, while appreciable quantities of hemicellulose may still be left (38, 35, 39).

A chemical analysis of lowmoor peats has shown that although the plants that contribute to the formation of these types of peat have a much greater percentage of cellulose than of hemicellulose, the peat which results from the anaerobic decomposition of these plants, contains very little cellulose but a considerable quantity of hemicellulose. Only a part of this hemicellulose is found to be pentosan; one lowmoor peat, for example, with 11.03 per cent hemicellulose, as determined by the reducing sugars formed on hydrolysis of the peat with dilute acid, contained only 2.52 per cent pentosan (35). This difference in the rapidity and nature of decomposition of the two types of carbohydrates is due primarily to two factors: *a.* True cellulose is a homogeneous complex and is decomposed completely by microörganisms at a uniform rate, provided the conditions for decomposition are favorable; the hemicelluloses represent a heterogeneous group of substances, some of which are readily attacked by microorganisms and others are more resistant to complete decomposition. *b.* In the process of decomposition of the organic matter by microörganisms, microbial cell substance is constantly synthesized by the organisms bringing about the decomposition processes; this cell substance contains considerable quantities of various hemicelluloses, commonly known as bacterial gums, slimes, capsular material, etc.

Egorov (7) demonstrated in 1911 that when manure undergoes decomposition in compost, the pentosans are attacked more quickly then the cellulose; however, toward the end of the decomposition period, the residual part of the compost contains more pentosan than cellulose. In view of the fact that the pentosan has usually been determined in these investigations by the furfuraldehyde yield obtained on boiling the material with 12 per cent HCl, it is more logical to conclude that, although the content of furfural-yielding substances is lower in the fresh manure than the content of true cellulose and that although those substances underwent a more rapid initial decomposition than the cellulose, there was left in the decomposed or "humified" manure more of the "furfural-yielding substances" than of the cellulose. This is probably a result of at least two distinct processes; the rapid initial decomposition is due to the destruction of the true pentosans, whereas the final accumulation of the resistant complexes is due to the resistance to rapid decomposition of certain uronic acid derivatives which also give furfuraldehyde on boiling with 12 per cent HCl.

Norman (22) has also shown that in the decomposition of straw by a mixture of microorganisms, there was at first a rapid loss of the major part of the hemicelluloses, followed by an equally rapid loss of the cellulose; however, after 12–14 days of decomposition, the hemicelluloses appeared to remain at a certain level, while the cellulose content continued to diminish progressively. This phenomenon was believed to be due to the non-homogeneous nature of the hemicelluloses, differing in the rapidity of their decomposition by microörganisms. The rapid initial loss in hemicelluloses is accompanied by a fall in furfuraldehyde yield, due to loss of the pentose groups. A later increase in these groups may take place, due to the formation of new compounds by the microörganisms; the uronic acid complexes in the straw appeared to be less readily decomposed than the true pentosans.

It is of further interest to note that whereas in the original plant material, the hemicellulose group is usually made up largely of pentosans, especially in the case of cereal straw and other residues, in the soil organic matter the hexosans may predominate over the pentosans.

The determination of pentosans in fresh plant materials, in composts, and in soil is usually based upon the furfuraldehyde yield obtained on boiling the material with 12 per cent HCl; the method of Kröber (14) is commonly employed for this purpose. Because of the fact, however, that uronic acids give on boiling with 12 per cent HCl a pentose and carbon dioxide, it is uncertain how much of the pentosan reported to be present in peat and in soil actually represents the polymer of pentoses and how much is due to the presence of uronic acid complexes in soil. Balks (2) has shown, for example, that although fresh pentosans added to the soil in the form of manures and plant residues decompose readily, the soil organic matter still contains 8.24 to 11.63 per cent pentosan. Methyl pentosans persist in the soil even longer. In general, the pentosan carbon was reported (27, 28) to make up 1.3 to 28.5 per cent of the total organic carbon in soil. Pentosans are especially abundant in peat, although the hydrolysis of peat with dilute acids was found to give, in addition to pentoses, also mannose, galactose, and levulose (10).

Most of the determinations of "pentosan" in soil and in composts probably fall short of the actual amount of total hemicellulose present. Since the pentose units yield only about 75 per cent of the theoretical amount of furfuraldehyde on distillation with mineral acids, since uronic acids give only 33 per cent of their weight as pentose, and further since hemicelluloses contain hexose units also, it is impossible to calculate the total hemicellulose from the determination of "pentosan" by the furfuraldehyde procedure (21).

The presence of uronic acids in soil humus has been recognized only during the last few years. The complexes containing uronic acid are brought into the soil in the form of plant residues and are abundantly synthesized by microörganisms. However, although the uronides in plant residues are made up largely of galacturonic acids, as in the case of fruit pectins, and to a less extent of glucuronic acids, the latter predominate among the microbial products, as in the capsular material of various bacteria.

Shorey and Martin (29) have recently shown that the proportion of uronic acid carbon to the total organic carbon of the soil ranges from 12.8 to 28.4 per cent in the case of mineral soils, and from 5.15 to 9.25 per cent in the case of peats. Only a small part of the uronides in soil will give furfural (12.5 to 35.0 per cent) as determined by the Kröber method, because of incomplete decarboxylation of the uronic acids by this treatment. In the case of peats, the relation between the furfural yield and the theoretical amount calculated from the uronic acid content of the material was 49.0 to 57.5 per cent. However, one should not necessarily consider this as due entirely to the insufficient hydrolysis and carboxylation of the uronic acid complexes. It may also be due to too high a figure obtained by calculating the uronic acid from the CO_2 liberated on hydrolysis. Norman and Martin (23) indicated that in the hydrolysis of pure pectin the CO_2 yield was higher than could be accounted for by the uronic acids present. The analysis of pectin reported in this paper seems to add some weight to this assumption. If this is true of pure pectin, it may certainly hold true of peat and especially of mineral soils, which contain a number of other complexes capable of liberating CO_2 on continued boiling with 12 per cent HCl, even if the carbonates are removed with dilute acid.

In a study of methods for determining the proximate chemical composition of the soil organic matter of soil humus, it was suggested (36) that the hemicellulose content be calculated from the amount of reducing sugar (multiplied by 0.9) obtained by hydrolyzing the soil with hot dilute mineral acids, under ordinary pressure, for several hours. It was found that 5.44 to 12.59 per cent of the total soil organic matter was made up of hemicelluloses. On comparing these results with the amount of pentosan present in the same soils, as determined by the regular furfuraldehyde procedure, it was found that the hemicellulose content obtained by the method of hydrolysis was considerably higher than the pentosan content. As a result of this, it was felt that the reducing sugar liberated from the soil organic matter on hydrolyzing the soil with hot dilute mineral acid at ordinary pressure is a more reliable index of the content of hemicellulose complexes in the soil organic matter than the total pentosan obtained by the furfural method. Because, however, the uronic acid complexes of the soil give reducing sugar on hydrolysis with hot mineral acids, investigations were undertaken, to obtain information which would enable one to correlate the total hemicellulose content of the soil, as measured by the production of reducing sugar, with the amount of uronic acid complexes in the soil organic matter; further, an attempt was also made to throw some light upon the origin of these complexes in the humus of composts and soils, formed under different conditions.

EXPERIMENTAL

In the following investigations, the determination of the total pentosan in the plant material and in soil was carried out according to the method of Kröber (14) as outlined by the Association of Official Agricultural Chemists. For the quantitative measurement of the uronic acids in the organic materials, the procedure of Lefevre and Tollens (15), as modified by Dickson, Otterson, and Link (6) was followed. This method consists in treating the material with an excess of 12 per cent hydrochloric acid, boiling (for 4 hours) until there is no further evolution of carbon dioxide, absorbing this gas in standard barium hydroxide solution, and titrating back with oxalic acid solution. In the case of plant substances, forest organic matter, and peat, 2-gm. portions of dry material were used for each determination, whereas in the case of inorganic soils, 25-gm. portions were employed. The hemicellulose determination was carried out by heating similar quantities of the organic substances with an excess of 2 per cent hydrochloric acid (150–200 cc.) for 5 hours, at ordinary atmospheric pressure, filtering, making up the filtrate to volume, neutralizing the acid, and determining the reducing sugar in an aliquot portion, by the Bertrand method. For the purpose of comparison, the plant substances, peat, and composts were analyzed by the proximate system of analysis, as outlined elsewhere (37).

Purified citrus pectin was employed for the purpose of comparing the three methods of determination of hemicellulose complexes. The following results were obtained:

	per cent
Uronic acid anhydride	76.5
Total furfuraldehyde	18.20
Pentosan equivalent	31.50
Hemicellulose sugar, obtained on boiling for 5 hours with 2 per cent HCl	50.7
Material insoluble on boiling with dilute acid	6.7

According to Norman, uronic acid anhydride gives 16.66 per cent furfuraldehyde. On this basis the furfuraldehyde equivalent of 76.5 per cent uronic acid anhydride would be 12.74 per cent. This leaves 5.46 per cent furfuraldehyde equivalent to 10.63 per cent of free pentose. Whether this free pentose is due to the presence of a free arabinose group in the pectin molecule, as shown by Ehrlich, or to some other factor remains to be determined. The sugar content obtained from the pectin is lower than the theoretical amount calls for, if all the uronic acid is converted by hot dilute acid to reducing sugar in this period of time. However, complete conversion does not take place, because a much longer period of boiling with dilute acid is required for this.

It is interesting to note that Ehrlich (8) reported an average of 72.5 per cent uronic acid in citrus pectin, whereas Dickson, Otterson, and Link reported for purified lemon pectin an equivalent of 72.60 per cent uronic acid anhydride. The reason for the incomplete yield of pentosan and reducing sugar from the uronic acid is to be looked for in the comparatively short period of time allowed for the treatment, and the weak acid used. The important point to be interpreted from these results is the fact that the hemicellulose sugar content approaches more nearly the actual uronic acid content than the pentosan determination commonly employed in the numerous analyses of plant materials, soils, and composts. In the case of materials, such as gum arabic, with only 15.83 per cent uronic acid anhydride, 80.93 per cent of the material was found as reducing sugar, calculated as glucose, on hydrolysis of the material with dilute acid.

TABLE 1

Hemicellulose and pentosan content of various "hemicellulose" preparations

On a dry and ash-free basis

SOURCE OF MATERIAL	HEMICELLULOSE CONTENT (REDUCING SUGAR × 0.9)	PENTOSAN CONTENT	URONIC ACID ANHYDRIDE
	per cent	*per cent*	*per cent*
Salep roots	80.8	2.40	1.32
Irish moss	63.0	2.3	1.26
Corn cobs	97.2	101.5	2.80

These results lead one to conclude that the sugar produced on hydrolysis of hemicelluloses with dilute acid does not give the theoretical amount of hemicellulose present; the greater the uronic acid content of the material, the greater will be the discrepancy obtained. However, the pentosan figure gives even less approximate results, unless we are dealing with a material that contains pure pentosans. This is shown in table 1. The xylan obtained from corn cobs gave the nearly theoretical amount, whether it was determined by the dilute acid hydrolysis or furfuraldehyde method (34). The preparations from salep roots and from Irish moss consisted largely of mannan and galactan respectively, and contained only small amounts of pentosan and uronic acids. The corn cob hemicellulose is thus found to represent a practically pure polysaccharide pentosan, giving a hundred per cent sugar on hydrolysis. Anderson (1) also found that corn cob hemicellulose contains only a small amount of uronic acid.

In order to study the fate of hemicelluloses, as measured by the various methods outlined, in the process of decomposition of plant materials by microorganisms, three plant materials were selected, varying distinctly in their nature and chemical composition; namely, oak leaves, rye straw, and sphagnum plants. These materials were available for analysis not only in a fresh state but also in a composted condition, the composts being about 3 years old (30).

TABLE 2

Proximate analysis of fresh and decomposed plant materials, used for pentosan and uronic acid studies

NATURE OF MATERIAL	CONDITIONS OF DECOMPOSITION	PER CENT OF DRY MATERIAL							
		Ether-soluble portion	Alcohol-soluble portion	Cold and hot water-soluble organic matter	Hemicellulose*	Cellulose	Lignin	Crude protein	Ash
Oak leaves.......	Fresh material	3.71	5.92	13.93	12.93	13.78	30.30	4.81	5.09
Oak leaves.......	Decomposed for 3 years	1.65	1.75	8.18	10.24	7.09	36.48	9.38	16.50
Rye straw.......	Fresh material	1.84	3.49	6.26	21.10	38.62	14.63	1.02	4.18
Rye straw.......	Decomposed 3 years, no added nutrients	1.10	1.33	3.24	19.96	27.67	24.88	3.94	8.50
Rye straw.......	Decomposed 3 years, with added nutrient salts	0 84	1.32	7.19	12.54	14.62	26.81	14.18	17.90
Sphagnum.......	Fresh material	1.11	....	6.95	27.73	19.21	7.33	5.50	.....

* Reducing sugar, obtained on boiling material previously treated with hot water, with 2 per cent hydrochloric acid at 100°C. for 5 hours, × 0.9.

In the case of some of the composts, nutrient salts including ammonium compounds and phosphates were originally added, which served to hasten the rapidity and extent of decomposition.

The proximate chemical analyses of the fresh plant materials and of the composts are given in table 2. The results show that with the aerobic decomposition of the plant materials there is a rapid decrease in the water-soluble substances, ether-soluble substances, and cellulose; a slower comparative decrease of the total hemicelluloses; a relative increase of the lignin; and an absolute increase of the protein. By using the increase in ash content as a measure of the extent of decomposition (the composts being kept in pots where no leaching took place), one can readily determine the absolute changes that have taken place in the concentration of the various chemical constituents of the plant materials as a result of decomposition.

The pentosan, total hemicellulose, and uronic acid figures for these materials are given in table 3. The results throw considerable light upon the comparative value of the different analytical procedures for the determination of the hemicellulose content in plant materials and in composts. In the case of the oak leaves, the total hemicellulose, as determined by hydrolysis of the material with hot dilute mineral acid and measuring the reducing sugars produced, is considerably higher than the pentosan content. The pentosans are readily hydrolyzed by hot dilute acids, as shown by the fact that the pentosan in the filtrate is as high as in the total untreated material. The high figure for the uronic acid anhydride content indicates that a large part of the pentosan in the oak leaves is in the form of polyuronides rather than as true pentosans. In the composted oak leaves, the uronic acid figure is higher whereas the pentosan figure is lower, showing that the free pentosans have largely decomposed, accompanied by a relative increase in the concentration of the uronic acid complexes. Table 2 shows that although the quantity of total material, as a result of decomposition, has been reduced to one-third of the initial material (as shown also by the relative ash increase), the cellulose content has dimin-

TABLE 3

Uronic acid and pentosan transformations in the decomposition of plant materials by microörganisms

NATURE OF MATERIAL	CONDITIONS OF DECOMPOSITION	PER CENT OF DRY MATERIAL				
		Total pentosan content	Uronic acid anhydride		Hydrolysis of total material by 2 per cent HCl	
			Method of Dickson, Otterson and Link	Method of Shorey and Martin	Total hemicellulose in filtrate (reducing sugar × 0.9)	Pentose sugar in filtrate
Oak leaves	Fresh material	11.70	11.53	9.74	20.10	11.80
Oak leaves	Decomposed for 3 years	7.10	12.30	9.81	10.90	5.50
Rye straw	Fresh material	28.50	4.58	3.59	25.00	22.30
Rye straw	Decomposed 3 years, no added nutrients	20.80	4.03	3.59	22.10	17.90
Rye straw	Decomposed 3 years, with added nutrient salts	10.50	4.61	4.32	14.00	9.20
Sphagnum	Fresh material	11.50	14.54	....	28.60	5.00
Sphagnum	Decomposed 3 years, aerobically	13.55	16.48	....	....	....
Sphagnum	Decomposed 3 years, anaerobically	12.80	15.47	....	....	.....

ished even more rapidly than the total material. The relative concentration of uronic acid has increased in a manner similar to that of the lignin; this tends to indicate that although the uronic acid compounds no doubt undergo a certain amount of decomposition by microorganisms, this process is slower than the decomposition of the cellulose and resembles more that of the lignin.

In the rye straw, the hemicellulose consists predominantly of pentosan. The uronic acid content is very limited, showing that this plant material contains largely the free polysaccharide pentosan. The amount of hemicellulose in the acid hydrolyzate was lower than the total pentosan; this is probably due to a certain loss of some of the pentose sugar during the prolonged boiling of the material with dilute acid, as is also indicated by the lower pentose content of the acid hydrolyzate.

With the decomposition of the rye straw, there is a drop in the total and relative pentosan content, especially in the presence of added nitrogen salts. The relative concentration of the uronic acid compounds, however, was not diminished to any great extent, proving again that this group of complexes undergoes a much slower decomposition than the cellulose and the true pentosans.

Sphagnum represents a type of plant material totally different in nature and chemical composition from the two previously considered. The total hemicellulose content of sphagnum plants was found to be considerably higher than the pentosan content, showing that the material is rich in hemicelluloses of a hexosan nature. The fact that the dilute acid hydrolyzate of the plants contains only about 40 per cent of the total pentosan found in the original plant material in the form of pentose sugar tends to indicate that either there was considerable loss of the pentose in the process of boiling of the material with the dilute acid or that not all the uronic acid complexes give pentoses on treatment with hot dilute mineral acid for a few hours, as brought out previously in the analysis of pectin. Actually the residual material, left after hydrolysis, was found to contain a considerable amount of uronic acid and gave furfuraldehyde on treatment with 12 per cent HCl. Further, the fact that more than 50 per cent of the sphagnum plant, as shown by loss in weight, is hydrolyzed by hot dilute acid and only 28–29 per cent is found as reducing sugar shows that a large part of the material is either volatilized, or not sufficiently hydrolyzed, or changed into non-reducing compounds.

With the advance in decomposition of the sphagnum plants there is no reduction in the uronic acid content but even an actual increase. This is accompanied by a parallel increase in the total pentosan content, tending to bring further evidence to show that most of the furfural obtained by boiling the sphagnum plants with 12 per cent hydrochloric acid is probably derived not from true pentosan but from polyuronides and other complexes.

In order to establish further the rôle of uronic acids in the process of decomposition of plant residues and their transformation into "humus," several peat materials and the organic matter of various horizons of two forest soil profiles were analyzed. The results are reported in tables 4 and 5. The two peat materials represent a lowmoor peat from Florida (Everglades region) and a highmoor peat from Maine. The lowmoor peat is characterized by practical freedom from cellulose; a very high lignin, protein, and ash content; and a considerable amount of hemicellulose; whereas the highmoor peat contains but little ash and protein, a fair amount of cellulose and lignin, and a considerable amount of hemicellulose.

TABLE 4

Proximate analysis of forest and peat material used for pentosan and uronic acid studies

NATURE OF MATERIAL	DEPTH OR LAYER	PER CENT DRY MATERIAL								
		Ether-soluble portion	Alcohol-soluble portion	Cold and hot water-soluble portion	Hemicellulose	Cellulose	Lignin	Ash	Crude protein	Total accounted for
Lowmoor peat.....	Everglades, Fla., 12–18-inch depth, fibrous layer	3.24	1.34	1.64	8.26	0.43	44.95	6.86	22.75	89.47
Highmoor peat.....	Orono, Maine, upper, younger peat, 1–4-inch depth	2.33	1.72	.....	23.85	14.52	23.16	2.28	4.82	72.68
Surrey spruce......	Litter	4.72	5.54	.....	15.67	16.31	34.91	3.04	5.81	86.00
Surrey spruce......	Humus layer	1.67	3.32	.....	9.19	2.36	30.50	26.40	10.44	83.88
White pine needles..	Fresh	6.08	3.34	12.74	20.88†	15.78	31.80	2.74	3.13	86.49
Pine forest soil*....	Litter	6.70	6.10	5.40	20.73†	13.70	35.00	4.00	6.10	‡
Pine forest soil*....	F layer	4.20	4.50	5.10	15.75†	8.20	45.90	13.00	10.60	‡
Pine forest soil*....	H layer	3.90	5.10	4.10	13.45†	5.20	49.50	19.00	11.10	‡
Pine forest soil*....	S H layer (A1)	4 60	4.50	4.70	11.76†	2.60	50.00	75.00	15.00	‡
Pine forest soil*....	Accumulation horizon (B1)	1.00	0.50	2.00	9.09†	1.10	32.30	91.20	36.20	‡

* Analyses of these materials reported on an ash-free basis.

† These analyses were made on separate portions of the fresh material.

‡ No total can be reported for these, since, in order to illustrate the changes in the organic matter content and because of the high ash content of the lower layers, the calculations were made on the ash-free material of this profile.

The relative concentration of total hemicellulose, pentosan, and uronic acid in these two peats is given in table 5. These results show that, in the case of the lowmoor peat, the amounts of pentosan and hemicellulose sugar produced on hydrolysis with dilute acids are not higher than the uronic acid content of the peat; provided all the uronic acid is hydrolyzed in that period of time to give reducing sugar, the peat is found to contain very little free pentosan but a certain amount of hexosan hemicelluloses.

The highmoor peat was found to contain a considerably larger amount of hemicellulose than of pentosan and uronic acid, showing that this material is rich in hexosan hemicelluloses. The fact that the amount of uronic acid found in this peat is considerable makes the presence of free pentosan in this material highly problematical, a fact already emphasized for sphagnum plants. Since certain polyuronides, as the citrus pectin, yield on hydrolysis with dilute acids only a part of the pentose that one might expect from theoretical calculations, one may be justified in concluding that the high uronic acid content of the sphagnum peat is sufficient evidence of the presence of substances not accounted for in the proximate analysis of this peat (35). It is of considerable interest to note that, although the total hemicellulose is somewhat lower in the sphagnum peat than in fresh sphagnum plants, the uronic acid content is higher, bearing out the same general principle observed previously that, with the advance of decomposition of plant material, there is a decrease in the total hemicellulose content but a relatively smaller decrease of the uronic acid complexes.

TABLE 5

Uronic acid and pentosan content of humus in peat and forest soils

NATURE OF MATERIAL	DEPTH OR LAYER	PER CENT OF DRY MATERIAL				
			Uronic acid anhydride		Hydrolysis by 2 per cent HCl	
		Total pentosan	Method of Dickson, Otterson and Link	Method of Shorey and Martin	Hemicellulose in filtrate	Pentosan in filtrate
Lowmoor peat	Everglades, Fla., 12–18-inch depth, fibrous layer	6.6	9.66	6.96	9.2	5.1
Highmoor peat	Orono, Maine, upper, younger peat, 1–4-foot depth	11.9	15.63	12.08	26.60	9.7
Surrey spruce	Litter	7.5	5.89	5.05	16.90	6.0
Surrey spruce	Humus layer	3.8	10.98	(5.64)?	9.60	2.9
White pine needles	Fresh	8.5	6.35	......	20.88	...
Pine forest soil*	Litter	9.79	6.96	......	20.73	...
Pine forest soil*	F layer	7.59	7.69	......	15.75	...
Pine forest soil*	H layer	5.74	7.56	......	13.45	...
Pine forest soil*	S H layer (A1)	4.48	9.20	......	11.76	...
Pine forest soil*	Accumulation horizon (B1)	2.27	32.39	......	9.09	...

* Analyses of these materials are reported on an ash-free basis.

In this connection, it may be of interest to call attention to the results of Keppeler (13), concerning the ratio of hexosan to pentosan in different peat materials of different age (taken from different depths of peat bogs). It was found that with the advance of decomposition of peat there is an increase in the hexosan-pentosan ratio, namely, from an average of 2.95:1, in the case of young sphagnum peat, to 4.1:1, in the case of old sphagnum peat. Although Keppeler included also the true cellulose among the hexosans, the fact that the cellulose is decomposed very readily and is found in diminishing amounts with an increase in age of peat would lead us to conclude that the increase in the hexosan-pentosan ratio is due to a more rapid destruction of the pentosans than of the hexosan hemicelluloses and uronic acid complexes.

One of the two forest soil profiles[2] was taken from an undisturbed forest soil at Keene, N. H., and one represents a Surrey spruce soil; of the latter, only the two organic matter layers above the inorganic soil were available. The proximate analysis of the various horizons of these soil profiles and the corresponding hemicellulose determinations are reported in tables 4 and 5. With the advance in the decomposition of the forest residues, there is a rapid decrease in the relative cellulose content and an increase in the ash, protein, and lignin content. The marked increase in protein in the accumulation horizon is due probably to the fact that the protein is washed down more readily than the non-nitrogenous complexes, a phenomenon already emphasized by Weis (40).

As to the changes in the relative concentration of the different hemicelluloses with the advance in decomposition of the organic matter, we find a rapid decrease in the total pentosan, a smaller decrease in the total hemicellulose content, and an actual relative increase in the uronic acid content. The exact quantitative relationship between the pentosan, total hemicellulose, and uronic acid content of different plant materials and especially of the various decomposition products is not fully known, because of the fact that the hydrolysis of uronic acid complexes does not give under these conditions the theoretical amount of reducing sugar and because different uronides contain different amounts of pentose sugar; a knowledge of the relative transformation of these three types of hemicellulose; namely, the total pentosan, the total hemicellulose, as measured by the amount of reducing sugar formed on hydrolysis of the material with hot dilute mineral acids, and the uronic acid content, enables us to interpret the behavior of these complexes in the process of decomposition of organic matter in the soil. The results so far obtained point definitely to the fact that although some hemicelluloses, especially the true pentosans, undergo rapid decomposition, other hemicelluloses, especially certain uronic acid complexes, decompose much more slowly. As a result of this, the total hemicellulose content may sometimes diminish rapidly in the process of decomposition of the plant materials in soils and in composts, as in the case of the rye straw, which is rich in true pentosans; at other times, the total hemicellulose may diminish very slowly, as in the case of the sphagnum plants rich in polyuronides; in still other cases, the hemicellulose transformation is irregular, as in the case of different forest materials, because of their varying chemical composition. This is the reason why different fungi and bacteria were found (26, 31) to decompose pentosans in different plant materials at a different rate, no doubt because of the different chemical nature of the "pentosans" in these plant materials.

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[2] The authors are indebted to Dr. Watson of this laboratory for collecting this material.

For the determination of uronic acids, the method of Shorey and Martin was compared, in some instances, with that of Dickson, Otterson, and Link. The first method gave invariably lower results, with undecomposed plant material, peat, and forest soils. This is because this method calls for boiling the material for one hour with 200 cc. of 1 per cent hydrochloric acid, in order to remove the carbonates. The fact that by this procedure, a considerably lower uronic acid content was obtained in the case of highmoor peat which has a pH of 4.0 and is practically free from carbonates shows that some loss of uronic acid-CO_2 takes place in the procedure recommended by Shorey and Martin. Hence the original method of Dickson et al., was employed in most other determinations.

TABLE 6

Pentosan and uronic acid content in soil organic matter

NUMBER OF SOIL	TREATMENT OF SOIL	CARBON CONTENT OF SOIL	ORGANIC MATTER IN SOIL	TOTAL PENTOSAN IN SOIL ORGANIC MATTER	HEMICELLULOSE IN SOIL ORGANIC MATTER (HYDROLYSIS BY 2 PER CENT HCl	URONIC ACID ANHYDRIDE IN SOIL ORGANIC MATTER	URONIC ACID CARBON, PERCENTAGE OF TOTAL ORGANIC CARBON
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	
5A	Manured	1.45	2.49	8.35	10.40	19.23	13.56
7A	Untreated	0.76	1.31	4.58	5.80	31.65	23.16
5B	Manured and limed	1.75	3.01	5.25	7.71	12.87	9.09
7B	Limed only	1.06	1.82	4.06	5.33	19.98	14.06

In order to establish further the relationship between the different hemicelluloses in humus of inorganic soils, which contain only a small amount of organic matter, a series of plots of a Sassafrass soil, variously treated for the last 20 years, were selected. As a result of the treatment of these soils with stable manure and lime, accompanied by cultivation and yearly crop growth, their organic matter content has become markedly modified both in quantity and kind. The plot receiving stable manure year after year (5A) contains nearly twice as much total organic matter as the unfertilized plot (7A). The plot receiving lime only contains more carbon than the untreated plot, because of the better crop growth and, therefore, of the larger amounts of plant residues left year after year in the soil. Liming of the soil resulted in a much greater reduction of the pentosan than of the total hemicellulose; although the limed soils contain more total organic matter than the corresponding unlimed soils, they contain less pentosan. The limed soils also contain less total hemicellulose and less of the uronic acid complexes, showing that the effect of lime upon the soil makes conditions favorable for the microorganisms capable of decomposing the more resistant hemicelluloses.

The fact that the uronic acid complexes are more resistant to rapid decomposition than cellulose and the true pentosans is brought out from the uronic acid figures. The unmanured plots were found to contain a much greater relative concentration of uronic acid complexes than the manured plots, this being true for both the limed and unlimed plots. In fact, nearly an inverse ratio was found to exist between the total amount of organic matter and the uronic acid content. The ratio for the total organic matter of the two unlimed plots (5A:7A) is equal to 1.9:1; the inverse ratio between the uronic acid content of the organic matter of these two plots is 1.7:1. The ratio of the organic matter content of the two limed plots (5B:7B) is equal to 1.65:1; the inverse ratio between the uronic acid content of these two plots is 1.55:1. This points further to the greater resistance to decomposition of the uronic acid complexes than of the other chemical constituents in plant residues; as a result of this, these complexes contribute an important fraction to the humus of the soil.

A knowledge of the relation between the different hemicelluloses is thus found to be of great importance not only for our understanding of the processes of decomposition of plant residues in composts, in peat, and in soil, but also for our understanding of the chemical nature of the humus of the soil. However, in attempting to measure these hemicelluloses quantitatively, at least four factors must be considered: *a*. In hydrolyzing the organic matter with mineral acids, incomplete transformation into sugar may take place, especially in the case of the uronic acid complexes, unless the boiling is prolonged. *b*. Under these conditions, some of the sugar may be destroyed or changed to furfural. *c*. The CO_2 obtained on boiling soil or peat with mineral acids may not come entirely from uronic acids, but from other organic and inorganic complexes. *d*. The hemicelluloses in soil humus, being made up of hexoses, pentoses, uronic acids, and possibly other groups, cannot possibly be measured by one single method; any one method will give only an imperfect idea of the abundance of the hemicelluloses.

SUMMARY

Under the term "hemicellulose," the authors include both the true hemicelluloses, as conceived originally by Schulze, and the polyuronides, according to the more recent conceptions of complexes which consist of mixtures of sugars and uronic acids.

In this paper a series of analyses are presented giving the abundance of pentosans, of total hemicelluloses, and of uronic acid complexes in plant materials, in composts where plant substances are undergoing active decomposition, in peats, in forest soils, and in mineral soils.

The results obtained show that in the decomposition of plant residues, the true pentosans are attacked more rapidly by microorganisms than are the uronic acid complexes. Whether these are as such more resistant to decomposition or whether they form compounds with the other chemical complexes as lignins and proteins, which renders them more resistant to rapid decomposition, still remains to be determined.

Different plant materials vary in the relative concentration of the different hemicelluloses and in the relative rapidity with which these are decomposed by microorganisms.

With the advance of decomposition of plant residues, there is a progressive diminution of the pentosans and a relative increase of the uronic acid complexes.

Manuring of soil results in an increase in the pentosan content, whereas continued cultivation of soil without the addition of fresh organic manures or plant residues, results in a rapid reduction of the total soil humus and a corresponding relative increase in uronic acid complexes.

The nature of the soil, its reaction, soil treatment, and system of fertilization will all modify not only the abundance of the total organic matter in soil, but also its chemical composition.

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THE EFFECT OF TEMPERATURE UPON NITROGEN FIXATION BY AZOTOBACTER[1]

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The Azotobacter group is widely distributed. The literature contains an abundance of citations of their uniform distribution throughout the entire United States. Lipman and Burgess (12) and others have shown Azotobacter to be present in a large number of foreign soils. The occurrence of Azotobacter in soils of Java is reported by Groenewege (5); in Japan by Yamagata and Itano (18); in India by Hutchinson (7); and in Denmark by Weis and Bornebusch (17).

When the great climatic variations between these countries are considered, the ability of Azotobacter to meet environmental conditions is remarkable. Thus it is not surprising that various investigators have studied the optimum conditions for nitrogen fixation by these organisms. Berthelot (2) was among the first to recognize that nitrogen fixation is influenced by temperature. He concluded that nitrogen fixation occurred best at summer temperatures, between 50° and 104°F., and that it was immediately stopped by heating soil to 230°F. Thiele (16) claimed that although Azotobacter could fix small quantities of nitrogen under laboratory conditions, these temperatures might not be favorable under field conditions. Heinze (6) showed that although the nitrogen-assimilating organisms were most active between 20° and 30°C., they were able to fix appreciable quantities of nitrogen at temperatures of 8° to 10°C. Koch (9) obtained fixation of 3, 11, and 15.5 mgm. of nitrogen per 100 gm. of soil incubated at 7°, 15°, and 24°C. respectively. Krzemeniewski (11) and Lohnis and Westerman (14) claim that the optimum temperature for nitrogen fixation is 28°C., and that extreme limits are 9° and 33°C. Lohnis (13) inoculated 100 cc. of 1 per cent mannite soil extract with 10 gm. of soil and incubated it at different temperatures; 3.15 mgm. of nitrogen were fixed at 10–20°C.; 4.55 mgm. at 20–22°C.; and 4.27 mgm. at 30–32°C. Jones (8) gives the maximum temperature for Azotobacter as 55–60°C., and the minimum near zero.

Soil microbiological investigations were commenced at the University of Arizona in the spring of 1930. As rapidly as time has permitted, soil samples have been obtained from the principal agricultural sections of the state. Most of the soils examined have possessed a very active nitrogen-fixing flora and Azotobacter have been found to be present in over 90 per cent of the soils examined to date.

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[1] Contribution from the department of agricultural chemistry, Arizona Agricultural Experiment Station. The author gratefully acknowledges the assistance and advice of Dr. P. S. Burgess, professor of agricultural chemistry, who suggested this problem.

The soils of southwestern Arizona are subjected to intense summer heat, and the soil temperatures are high. In the first few inches of the soil, the temperature during the summer months is high enough to coagulate egg albumin. Children at play, often bury eggs in the surface soil, and the eggs are "baked" within a short time. Although there is probably no fixation in the first inch or so, the lower depths of soil are also subjected to fairly high temperatures. Unpublished results of Evans[2] show that the average annual temperatures of soil in the experimental plots on the university campus are approximately 29°C. at a depth of 4 inches, and 21°C. at 10 inches. In the summer months these temperatures are somewhat higher probably attaining a maximum of approximately 32°. at 4 inches.

The purpose of this paper is to compare the amount of nitrogen fixed by the known species of Azotobacter at different temperatures with the quantities fixed by species isolated from Arizona soils, under similar conditions.

EXPERIMENTAL

In the summer of 1930, some preliminary tests were made to determine the effects of temperature upon nitrogen fixation. One hundred cubic centimeters of Ashby's liquid medium was inoculated with 2 gm. of soil, incubated at various temperatures for 14 days, and then analyzed for total nitrogen by the modified Kjeldahl method. The results, which are given in table 1, show that there was considerable fixation at temperatures higher than those generally considered optimum. It was then decided to isolate the Azotobacter species and to compare their azofying power with those of pure cultures of the various species of Azotobacter.

The six species of Azotobacter described by Bergey (1) were obtained from culture collections (three from the American type culture collection and three from Dr. E. Pribram's Mikrobiologische Sammlung, Vienna); and the local species were isolated from soils which had shown an especially active Azotobacter flora by inoculating 1 gm. of soil into 100 cc. of sterile Ashby's liquid medium which contained a small excess of calcium carbonate. The flasks were incubated for several days at 28°C. and a loop of the film was transferred by a sterile inoculating needle to a sterile flask of tap water. Dilutions were made and plates were poured, using Ashby's agar medium. The plates were examined both macroscopically and microscopically. Typical Azotobacter colonies were transferred to Ashby agar slants and incubated.

Slides were prepared and the stained mounts were examined carefully to detect any contaminating organisms. It was necessary to replate some of these cultures several times in order to remove contaminating organisms. Seven pure cultures were obtained and were used in these experiments.

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[2] Evans, M. M., research assistant in plant pathology, University of Arizona. Private communication.

No attempts were made to identify these organisms. *Azotobacter chroococcum* was probably the most common form. Each culture was designated by a number, corresponding to the soil sample from which it was isolated.

Cultures 3-1 and 3-4 were isolated from cultivated soil near Tucson; 5A and 6B from a cultivated soil several miles west of Tucson, which contained a rather large amount of soluble alkali salts; 13 was isolated from a virgin soil in the Casa Grande Valley, 1½ miles northeast of Casa Grande; 50-C and 50-4 from a reddish sandy soil in Oak Creek Canyon, about 75 miles northeast of Prescott (Yavapai County).

Twenty-four hours prior to inoculation, the stock cultures were transferred to slants of Ashby agar containing 0.05 per cent KNO_3, and incubated at 28°C. At the end of 24 hours the slants were completely covered with growth. The cultures were examined microscopically before each inoculation. A small amount of these actively growing cultures were transferred to 10 cc. of sterile tap water, and after being shaken gently, 1 cc. of this suspension was used as an inoculum. Fuller and Rettger (3) have shown that the size of inoculum is unimportant as long as a sufficient seeding is obtained to give satisfactory growth.

TABLE 1

Nitrogen fixed by mixed soil cultures at different temperatures

(100 cc. Ashby's solution inoculated with 2 gm. soil)

SOIL NUMBER	10°C.	28–30°C.	35°C.	40°C.	45°C.	50°C.
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm*
I C	2.7	14.7	10.0	4.9	3.2	2.2
5 A	1.2	16.0	14.0	2.2	1.3	1.2
6 C	1.7	15.0	14.5	6.1	4.4	1.6
10 C	1.6	13.3	10.9	7.9	2.8	1.6
15	2.6	15.0	12.5	11.4	3.8	3.6
19 A	1.0	13.0	5.6	4.3	5.0	1.0

In order to approximate soil conditions to a certain extent and to promote aeration without the difficulties of using sterilized soil, sand slants were used in these experiments. Krainskii (10) found that cultures of Azotobacter, when grown in Ashby's solution containing sand, fix two to three times as much nitrogen as do the same cultures growing in Ashby's solution. Greaves (4) cites similar increases when gypsum or soil is added to Ashby's medium; the increases are attributed to greater aeration in the cultures, and probably to colloids added.

Sand, which had been previously washed with steam, was washed several times with water, dried and sterilized in a hot air oven at 200°C. for 4 hours. One hundred grams of this sand was placed in a 500-cc. Erlenmeyer flask with

100 cc. of Ashby's solution and sterilized at 15 pounds for 15 minutes. When cool, these flasks were inoculated with 1 cc., of the bacterial suspensions, and

TABLE 2

Nitrogen fixed by pure cultures of azotobacter at different temperatures

(1 cc. of aqueous suspension inoculated into 100 cc. of Ashby's solution)

CULTURE	18°C.	23°C.	27.5°C.	30°C.	32.5°C.	35°C.	37.5°C.	40°C.	42.5°C.
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
A. agilis	6.0	11.1	11.6	11.3	12.6	10.5	4.9	2.0	1.6
A. vinelandii	3.5	7.2	9.6	11.5	10.8	8.8	2.9	0.3	0.2
A. vitreus	0.9	2.0	1.8	1.9	1.6	1.3	1.3	1.2	0.2
A. woodstownii	0.5	1.6	2.1	2.9	1.2	0.9	1.8	0.6	0.1
A. beijerinckis	1.9	5.1	8.1	7.4	8.3	1.5	1.0	0.5	0.6
A. chroococcum	0.9	3.5	5.4	6.1	9.0	8.9	1.7	2.8	0.5
3-1	0.5	9.2	8.9	10.5	11.4	9.2	10.4	9.3	0.9
3-4	1.4	7.4	9.2	8.7	10.5	9.2	9.3	10.2	0.5
5-A	0.4	7.6	9.7	10.4	11.2	9.1	9.5	9.3	0.6
6-B	0.4	7.5	8.4	9.9	10.3	9.5	9.4	8.7	0.2
13	0.6	6.1	10.4	10.9	9.9	11.8	7.7	5.8	0.2
50-C	1.6	6.3	9.3	9.5	11.5	10.6	9.1	10.4	0.2
50-4	1.4	6.9	8.7	8.6	10.5	8.7	6.5	3.2	0.2

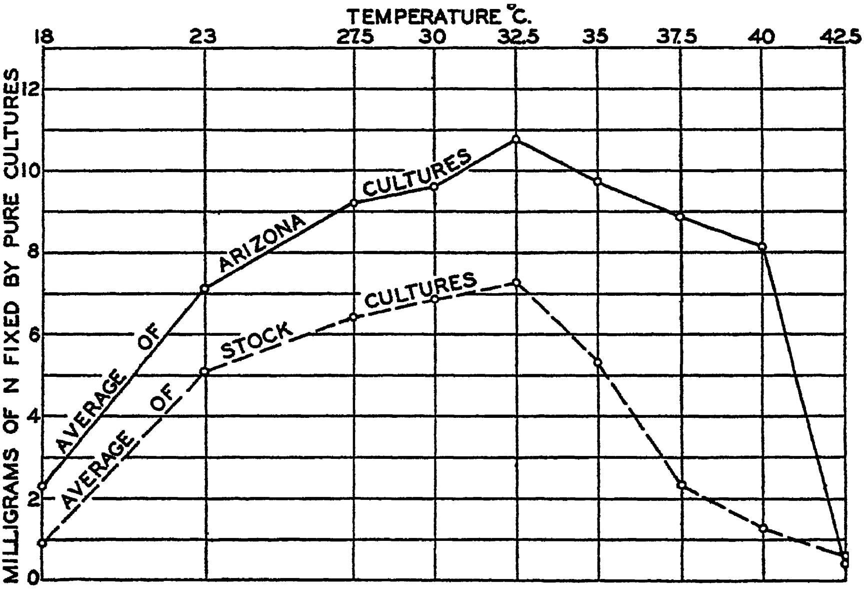

FIG. 1. THE EFFECT OF TEMPERATURE UPON NITROGEN FIXATION BY AZOTOBACTER

approximately 1 gm. of sterilized $CaCO_3$ was added. The flasks were whirled gently and then inclined at an angle of about 40°. In this manner a slant of sand was formed which extended well above the surface of the liquid. Two flasks were inoculated from each culture and the flasks were incubated at the temperatures given in table 2.

The period of incubation was arbitrarily set at 14 days. Preliminary tests showed that at 28°C. most of the mannite was used up within a period of less than 8 days, and although larger amounts of nitrogen night have been fixed in a longer period of incubation, it was concluded that, in such a comparative study as this, 2 weeks would be sufficient time for incubation.

At the end of 14 days the cultures were carefully examined, and their appearance was noted (presence of film, pigment formation, etc.). The cultures were then shaken vigorously, allowed to settle a few seconds, and the solution was decanted to an 800-cc. Pyrex Kjeldahl flask. Thirty-five cubic centimeter of concentrated H_2SO_4 was added to each flask, and allowed to stand for 30 minutes with frequent agitation. The acid was then decanted into the Kjeldahl flask, and the sand remaining in the flasks was washed with 500 cc. of distilled water, using 50-cc. portions of water, shaking well, and then decanting off the liquid.

The excess of water was boiled off, and heating continued until charring commenced, then Hibbard's digestion mixture was added, and nitrogen was determined in the usual manner by the Kjeldahl method. In the first few series, blank determinations were made upon cultures inoculated in the same manner as the flasks which were incubated. The amount of nitrogen added in the inoculums could not be detected by the Kjeldahl method. In the succeeding series, blank determinations were made upon 100 cc. of the media only. The results of the analyses, from which the blank (0.1 mgm. per flask) has been deducted, are given in tables 1 and 2 and figure 1.

DISCUSSION

Tables 1 and 2 reveal several interesting facts. Table 1 shows that these soils have a very active nitrogen-fixing flora. The amounts of nitrogen fixed at 45° and 50° are significant, although it is doubtful whether fixation at these temperatures was due to Azotobacter, since film formation, which is characteristic of Azotobacter, was very slight at 40° and entirely lacking in some cases.

There is a great difference between the quantities of nitrogen fixed by the various Azotobacter stock cultures. Of these, *A. agilis* was by far the most active fixer, with *A. vinelandii* a close second; *A. beijerinckii* grew well from 27 to 32.5°C., and *A. chroococcum* was most active between 30 and 35°. It is also interesting to note that the greatest quantity of nitrogen was fixed at 32.5°, and that *A. agilis*, *vinelandii*, and *chroococcum* fixed considerable amounts at 35°, although the optimum temperature is considered to be 28°. *A. woodstownii* and *vitreus* were not active fixers at any temperatures.

The cultures of Azotobacter isolated from Arizona soils fixed less nitrogen at 18° than the stock cultures, but in the succeeding temperatures, the amounts were much greater. This is especially true at the higher temperatures. At

37.5° and 40° fixation is still very active, although the latter temperature is apparently the maximum for fixation under the conditions of these experiments.

There was not a great deal of variation in the amounts of nitrogen fixed by the Arizona cultures, as compared with the stock cultures.

It might be well for purposes of comparison to evaluate the temperatures used in terms of mean average temperatures of some cities. Smith (15) gives such a comparison. The mean annual temperature of Athens, Greece; Ft. Worth, Texas; Tucson, Los Angeles, and Buenos Aires is approximately 17–18°C. (63–65°F.); for Brawley, California, Mohawk, Arizona, and Rio de Janeiro, approximately 23°C. (73–75°F.); Yucatan and Khartum have mean annual temperatures of about 26°C. The record maximum for the United States is 57°C. (134°C.) in Death Valley.

TABLE 3

Average mean monthly temperatures of certain cities

CITY	JANUARY	FEBRUARY	MARCH	APRIL	MAY	JUNE	JULY	AUGUST	SEPTEMBER	OCTOBER	NOVEMBER	DECEMBER	ANNUAL
	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.
Boston	−2.2	−1.6	2.2	7.7	13.7	18.8	22.2	21.0	17.1	12.1	6.0	0	9.8
Chicago	−3.9	−2.8	2.2	8.8	14.3	19.9	23.3	22.7	18.8	18.2	4.9	−1.1	10.3
Sioux City	−7.7	−6.1	0.5	8.8	15.6	21.0	23.3	22.2	17.1	10.4	1.6	−5.0	8.2
Kansas City	−2.2	−0.6	6.0	12.6	18.2	23.3	25.5	25.0	20.4	14 3	7.1	0	12.3
Seattle	4.4	4.9	7.1	9.3	12.1	14.8	17.1	17.1	14.3	10.4	7.7	5.5	10.4
Maricopa, Ariz.	9.9	12.6	15.6	20.4	25.5	30.8	33.8	32.7	28.8	21.5	15.6	10.4	20.2
Prescott, Ariz.	0.2	3.5	6.3	9.5	13.2	18.2	22 2	21.0	17.7	11 5	6.6	1.9	11.1
Tucson, Ariz.	9.9	11.5	13.8	17.4	21.8	27.3	29.4	28.5	26.3	20.4	14.0	9.9	19.2
Yuma, Ariz.	12.4	14.7	17.6	20.9	24.6	29.1	32 6	32.5	28.9	22.5	17.3	12.6	21.9
Jerome, Ariz.	5.7	7.4	10.0	13.8	18.2	24.5	25.9	24.4	20 4	16.5	11.1	5.8	15.4

Three of the stock cultures were obtained from the northern part of the United States, and three from Vienna, where the mean annual temperatures are approximately 10° and 8°C., respectively, while four of the Arizona cultures were isolated from soils near Tucson, where the mean annual temperature is 18°C., and the extreme maximum and minimum are 45°C. and −14°C.; one from near Casa Grande where the mean annual temperature is slightly higher, and the extremes are 50° and −8°C. (112–17°F.). The two remaining cultures were isolated from soils in the lower Oak Creek Canyon, about 75 miles northeast of Prescott. Temperatures are not available for this district but the mean annual is somewhere near 17°C., with extremes of 45° to −14°.

Table 3 shows the average mean monthly temperature of certain cities of Arizona and other states. These figures, taken from Prof. Smith's bulletin (15), have all been converted to degrees Centigrade.

The temperatures are given for Maricopa because mean monthly average figures were not available for Casa Grande. The altitude of Prescott is 5,008 feet, which accounts for the lower temperatures. The temperature of Jerome, however, is nearer to that of Oak Creek Canyon, where samples 50-C and 50-4 were taken. The averages for Tucson and Yuma give some idea of the temperatures to which the soils of southern Arizona are exposed. It must be remembered that these are mean temperatures, and not extremes, which have been as great as 44.2°C. at Tucson, and 47.9°C. at Yuma. Table 3 shows that with the exception of Prescott, the temperatures of the Arizona cities are considerably higher for every month than the temperatures of the other cities.

It is apparent that the Azotobacter flora of Arizona soils have been subject to high temperatures for many thousands of years. In southern Arizona the lowest mean extreme maximum temperature, which is for the month of January, is 26°C., whereas during the months of May to September the mean extreme maxima vary from 37° to 41°.

It is not surprising, therefore, that these organisms are more active nitrogen fixers at high temperatures than are the stock cultures which were isolated from soils of more temperate regions. On the other hand, neither is it surprising that the latter were more active at lower temperatures. It does not seem, therefore, too far fetched to consider that these organisms have adapted themselves somewhat to their particular environment.

Objections may be raised to this assumption, but the fact remains that the stock cultures which were isolated from soils of temperate regions and which had been cultivated artificially for many generations on synthetic media at a temperature of 28°, far above the average temperature of the soils from which they came, did not fix as much nitrogen at higher temperatures as did those from Arizona soils, which are accustomed to higher temperatures.

Another objection is the possible loss of vigor due to prolonged cultivation under artificial conditions. Indeed, Fuller and Rettger (3) show that a freshly isolated strain of *Azotobacter chroococcum* fixed more nitrogen than did three stock strains of the same organism. This difference however, is not so great as the differences between the amounts of nitrogen fixed by the Arizona cultures and the stock cultures. Although it is possible, under certain conditions to attenuate some organisms, it is not possible to affect others materially. The typhoid organism may remain in a dormant state for a considerable period of time in ice cream, and yet a most virulent case of typhoid may result from some person eating that food. The Arizona cultures were isolated in October, 1930, and have been cultivated continuously on synthetic media since that time. These fixation experiments were not commenced until February, 1931, and were continued until August, 1931. Thus, any unusual vigor of a freshly isolated culture was avoided.

The life cycle of Azotobacter is short, but it has probably been so for ages. It does not seem possible, therefore, by means of cultivating these organisms for a relatively short time under entirely different conditions, to obliterate the effect of an environment that has lasted for a long period of years.

SUMMARY

The physiological activity of the six recognized species of Azotobacter has been determined at different temperatures. *A. agilis* is the most active nitrogen fixer, with *A. vinelandii* a close second. *A. beijerinckii* and *A. chroococcum* are next in order. *A. vitreus* and *A. woodstownii* were not active fixers at any temperatures.

The seven cultures of Azotobacter isolated from Arizona soils fixed greater quantities of nitrogen at all temperatures except at 18.0°C.

In general, greatest fixation occurred at 32.5°C. *A. agilis, vinelandii,* and *chroococcum* were quite active at 35°. Although the cultures from Arizona soils fixed the greatest amount of nitrogen at 32.5°, they were very active at higher temperatures. The quantities of nitrogen fixed by them at 40°C. were approximately the same as those fixed at 27.5°C. The maximum temperature for fixation was 40°C.

The Azotobacter flora of Arizona soils have been subjected to high temperatures for a long period of time. Data presented in this paper indicate that they have become adapted to this environment, and consequently are able to fix nitrogen at higher temperatures than cultures of Azotobacter which come from soils of more temperate regions.

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SORPTION PHENOMENA IN SOILS AND IN SILICA GELS

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The obscurity of many absorption and adsorption reactions, especially those occurring in soils, has led to a certain confusion in the use of terms: for this reason and to avoid the implication of any precise physical interpretation, the word "sorption" is employed in the present paper.

An attempt has been made to use conductivity measurements on soil-in-water suspensions to give a general and relative idea of the sorptive capacities of soils, correlating these observations with pH determinations. A comparison of natural soils was followed by observations on "hydrogen soils," on "calcium soils," and on pure silica gels in order that the specific effects of different ions might be compared and their part in the sorption mechanism elucidated. Similar determinations were made with soils previously heated through various ranges of temperature in order that further information (3) on the nature of changes thereby produced might throw light upon the process of sorption itself.

EXPERIMENTAL DETAILS

Conductivity measurements

The comparatively high resistances (specific conductivity of the order of 10^{-4} mhos) of soil in water suspensions (1 gm. soil: 5 cc. water) necessitated the use of large electrodes placed fairly close together; and, since stirring was necessary to ensure attainment of equilibrium, the electrodes had to be fixed securely in order that their relative position should remain unaffected. This condition was fulfilled by having small rods of borosilicate fastened across the corners of the electrodes, as shown in figure 1. Stirring was effected mechanically, using a stirrer of Pyrex glass made to move up and down outside the electrodes.

A number of Jena beakers served as conductivity cells: their rims were ground so as to give an air-tight fit with ground glass covers when not actually closed by the mahogany rubber-lined cover holding the electrodes. The whole arrangement is shown in figure 2.

All the conductivity measurements were taken at 18°C. The conductivity beakers were steamed before use. The cell constant (0.094) was checked from time to time, and remained invariable throughout the series of measurements.

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[1] The author wishes to thank Mr. C. G. T. Morison, in whose laboratory the work was carried out and whose criticism and advice were invaluable.

The water used for conductivity and pH measurements was purified by boiling out distilled water in the Jena flasks normally used for storage, closing these before cooling with corks fitted to soda-lime tubes: It had an average specific

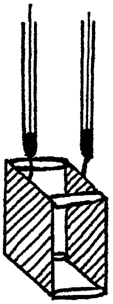

Fig. 1. Rods of Borosilicate Fastened Across the Corners of the Electrodes

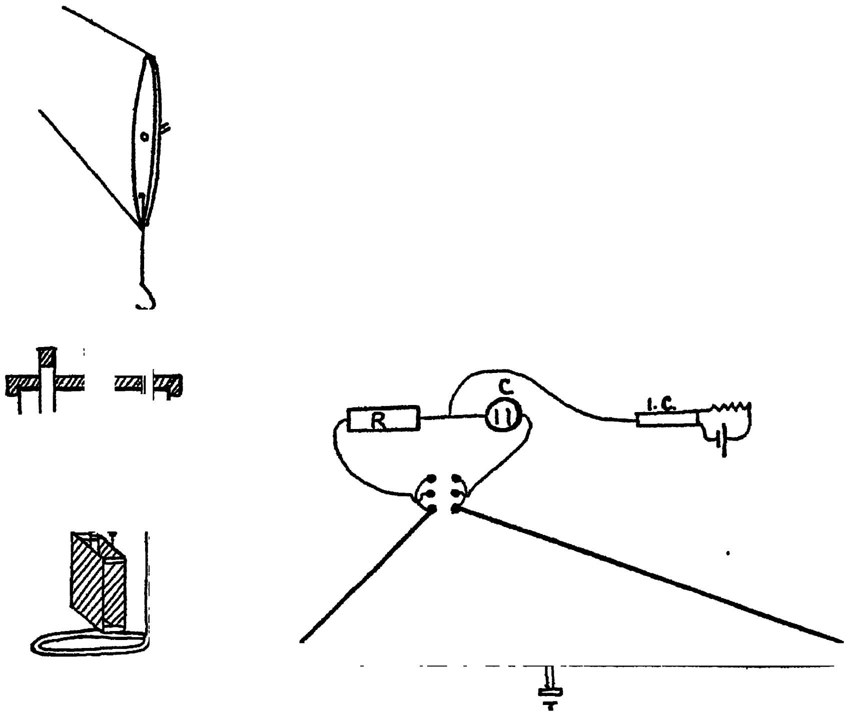

Fig. 2. Arrangement of Conductivity Cells

conductivity of 1.6 × 10^{-6} mhos. All the salts used for sorption measurements were standardized conductometrically.

The 1:5 aqueous suspensions were made up in the proportion 30 gm. soil: 149.8 gm. (150 cc. at 18°C.) water, and stood in a thermostat at 18°C. It was observed that mechanical stirring seldom altered the conductivity of suspensions which had been allowed to settle out overnight; and, in conductivity changes due to sorption, it was only necessary to stir for several minutes before an equilibrium value was attained. For the experiments on heated soils, duplicate 30-gm. lots were placed in weighed silica basins and heated in ovens at different temperatures: a few hours' heating to redness was sufficient to bring to constant weight but at 98°C. the weight altered gradually for several days and the heating was continued for a week. In order to obtain more strictly comparable results for soils which had lost a certain amount of water on heating, the aqueous suspensions were always made up with water to a weight of (30+149.8) gm., the excess water being allowed for in calculating conductivity due to added salts.

The conductivity of unheated soil-in-water suspensions showed a continuous drift, probably on account of bacterial action. Concordant initial values which remained constant over varying periods were attained by boiling the suspensions for a short time before use. The standard method finally adopted was to add to 30 gm. soil approximately 155 cc. water (to a mark on the outside of the beaker), bring quickly to the boil, keep boiling for 10 minutes, cool, and make up to a weight of (30 + 149.8) gm. This seemed to be the most accurate method attainable since it prevented bacterial action and, at the same time, gave values differing but slightly from those of suspensions kept at 18°C.

pH measurements

PH measurements and pH titrations were made on 1:5 aqueous soil suspensions using the quinhydrone electrode of Biilmann (1). Equilibrium pH values were reached almost instantaneously.

Determination of the "degree of unsaturation" (T-S)

The method of Hissink (5) was employed. Two-gram portions of soil were shaken with 50 cc. baryta solution of different initial concentrations and the final concentration of alkali was estimated after standing by titrating samples of the supernatant solution.

According to Hissink, the sorption of base from solution requires several days to reach equilibrium. On employing mechanical shaking, however, it was found that equilibrium hydroxyl concentrations were reached almost at once. Attainment of conductivity values which remained constant after the first 10 minutes on addition of KOH confirmed this conclusion.

Electrodialysis of suspensions

The dialysis cells were of the type designed by Mattson (8). The parchment membranes used in the first series of experiments were later replaced by cellophane paper. The electrodes were made as large as possible in order to minimize heating effects: the cathodes were made of fine nickel gauze and the anodes of platinized glass plates, made by fusing colloidal platinum into glass at its melting-point. The P.D. across the cells was 100 volts and the maximum current about 0.2 amps.

The anode and cathode solutions were replaced after increasing intervals of time by distilled water. The total exchangeable cations were estimated as hydroxides, after addition of excess of standard acid, by titration. The anode dialyzates were titrated directly with 0.01 *N* KOH using methyl red as indicator: the total anions estimated in this way were only about one-eighth of the total cations: qualitative tests showed the presence of carbonate, nitrate, and sulfate; but no silicate ions could be detected in the anode solutions and were apparently unable to pass the pores of the parchment membrane.

It was found that small quantities of anions and cations continued to dialyze after an indefinite period of time; even two months of electrodialysis did not completely obviate further extraction.

The suspensions remaining after electrodialysis were washed into conductivity beakers and boiled as in the usual method. They were all extremely sticky: they had a pH of about 3.8 and a specific conductivity of about 0.5×10^{-4} mhos.

Five typical subsoils were employed. Their general characteristics are shown in table 1.

If no sorption or exchange reactions or other gel effect obtained, the specific conductivity of a suspension in *dilute* aqueous suspension should be the sum of two values:—the original specific conductivity and that of $x/(150 + x)$ *N* chloride solution, where x = cc. *N* chloride added to 150 cc. solution. Any variation from this calculated value would be due to the soil- or gel-complex.

After obtaining conductivity curves for addition of the salts to water only, preliminary tests were made with filtered soil and gel *extracts*. The calculated conductivity values were found to be identical with those obtained experimentally.

But, in cases of soil *suspensions*, a decrease in specific conductivity from the calculated value together with an increase in the acidity of the resulting solution was observed. This indicates that there is some kind of sorption from solution much greater in extent than the exchange reactions postulated by Page (10) and others. If the only reaction was a hydrogen exchange of the type MCl + H complex = HCl + M complex, the conductivity of the solution would increase, whereas exchange with other cations would have comparatively little effect. The decrease in conductivity due to sorption is therefore greater than that actually observed by an amount corresponding to the hydrion exchange.

The conductivity value observed about 10 minutes after each addition of chloride solution remained constant over periods varying from a few hours to several days, showing that the sorption, displacement, or whatever reaction it may be, is completed immediately.

Changes in specific conductivity and in pH on addition of various chloride solutions to all five soils are shown in table 2. It is seen from the table that, of the salts added, aluminum chloride gave the lowest final pH values; but, when calculations were made to deduct acidity due to the salt solution itself, an actual decrease in hydrion concentration was found. Of the other salts added, displacement of hydrion was found to be greatest in the order Ca > Mg and K > Na.

TABLE 1

Characteristics of the five subsoils used

SOILS	CLAY	$CaCO_3$	(T-S)	pH	SPECIFIC CONDUCTIVITY
	per cent	*per cent*	*m.e. per 100 gm.*		*mhos* $\times 10^{-4}$
I	33	...	32	4.6	1.40
II	56	...	36	7.5	2.81
III	9	0.1	15	7.8	1.03
IV	7.5	9.9	15	7.6	1.96
V	3.4	1.6	5	8.5	0.70

TABLE 2

(B-A) values and final pH values for addition of chlorides to aqueous soil suspensions

A = observed specific conductivity in mhos $\times 10^{-4}$.
B = calculated specific conductivity in mhos $\times 10^{-4}$.
N = normality of salt in mille-equivalents per liter.

N	2.5	5.0	7.5	10.0	pH	2.5	5.0	7.5	10.0	pH
	NaCl					KCl				
I	0.39	0.74	1.48	1.76	4.2	0.77	1.47	2.55	3.37	....
II	0.52	0.96	1.75	1.95	5.8	0.80	1.62	2.60	3.95	5.8
III	0.41	0.88	1.55	1.95	6.7	0.85	1.39	2.32	3.22	....
IV	0.47	1.03	1.82	2.30	7.2	0.65	1.26	2.14	2.76	....
V	....	0.80	1.15	1.33	7.8	0.58	1.15	2.07	2.71	....
	$MgCl_2$					$CaCl_2$				
I	0.32	0.52	0.88	1.39	4.0	....	....	....	1.69	4.0
II	0.35	0.64	1.06	1.51	5.7	0.70	1.22	2.0	2.88	5.5
III	0.50	0.88	1.29	1.78	6.5	....	....	.....	2.0	6.3
IV	0.47	0.82	1.24	1.65	7.1	....	.....	.....	2.32	6.9
V	0.35	0.70	1.14	1.69	7.1	....	....	.....	1.16	7.5
	$AlCl_3$					HCl				
I	....	0.98	....	2.31	....	6.4	11.4	15.7	.....	3.33
II	....	1.22	....	2.86	5.25	7.4	14.8	22.5	30.1	4.51
III	....	1.50	....	2.92	5.36	7.4	14.8	22.1	29.7	4.52
IV	....	0.66	....	1.79	6.8	7.0	14.2	21.5	28.8	6.55
V	....	0.68	....	0.4	7.1	5.8	12.7	19.8	27.0	7.14

When the conductivity decrease is plotted against calculated concentration of added salt, regular curves are obtained. Soil II, having the highest clay content and showing generally greatest sorption, is taken as the typical soil for comparison of conductivity decreases in the different chloride suspensions. Figure 3 shows $(B\text{-}A)^v$ concentration curves for salts added to soil II.

In the subsequent comparison of sorptions for ordinary soils, no allowance is made for the slight increase in acidity resulting from these additions, since the (B-A) values are at least one hundred times as great as the increases in conductivity due to hydrion exchange. (The decrease in pH is generally from about 7.5 to 5.5 in 0.01 *N* salt solution: this is a hydrion increase of only

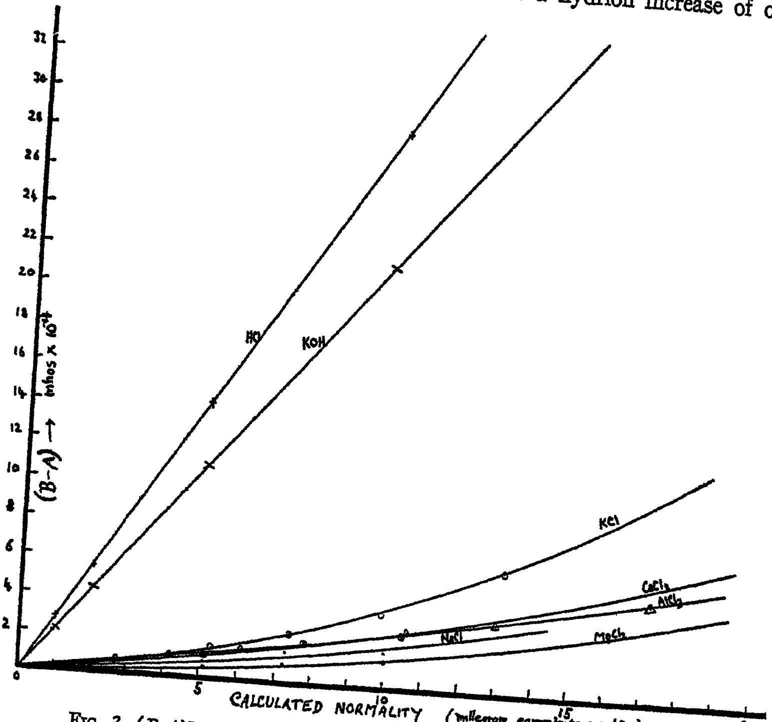

FIG. 3. $(B\text{-}A)^v$ CONCENTRATION CURVES FOR SALTS ADDED TO SOIL II

0.003 m.e. per liter, whereas the cation decrease, as noted from change in conductivity, is approximately 2 m.e. per liter.)

Neglecting exchange and therefore assuming equivalent sorption of anion and cation, the apparent decrease in salt concentration can be read off directly from suitable graphs of specific conductivity of aqueous salt solutions once the (B-A) values are known. The results of this process for soil II are shown graphically in figure 4, from which it would appear that the cations have specific effects on the sorption process.

Table 3 gives a comparison of sorptions for the other soils as well: the order of sorption for soil II is not exactly the same as that for the other soils, and must obviously be influenced by the exchangeable bases already present in the soil. In order to find the extent of this influence, a number of "hydrogen,"

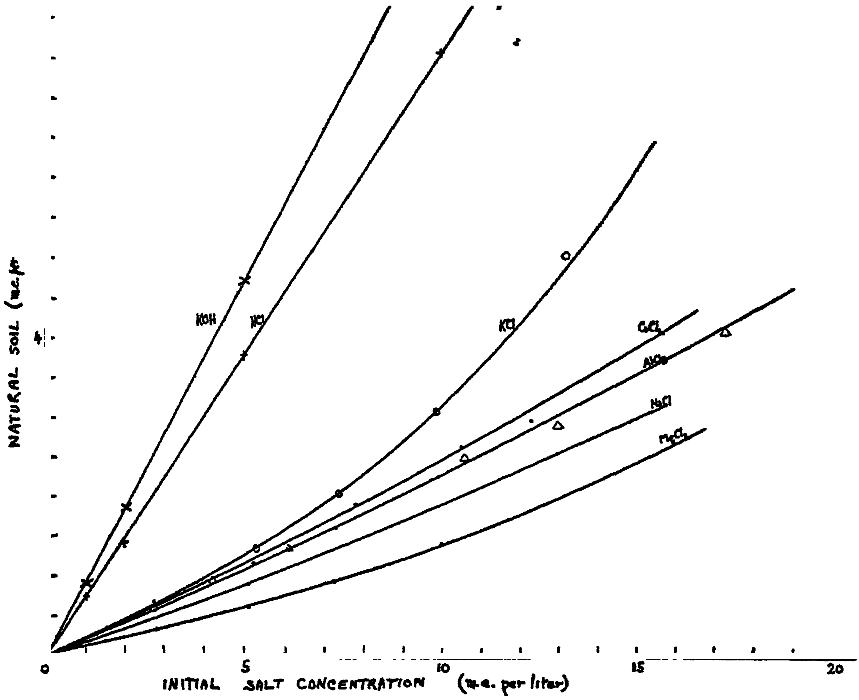

FIG. 4. SORPTION VALUES FOR A NATURAL CLAY SUBSOIL (SOIL II)

Explanatory note: The values plotted against *concentration* in this figure are *not* conductivity values: they are the actual *amounts* in m.e. per liter of the ions sorbed.—AUTHOR.

TABLE 3

Sorption values in m.e. per liter for solutions originally 0.01 N with respect to the salt added

SOIL	HCl	NaCl	KCl	$MgCl_2$	$CaCl_2$	$AlCl_3$
I	7.20	1.66	2.68	1.32	1 54	1.83
II	8 01	1.85	3.13	1.44	2.62	2.26
III	7.91	1 85	2.58	1.70	1.82	2.32
IV	7.67	2.19	2.19	1.58	2.11	1.42
V	7.20	1.26	2.15	1.62	1.05	0.31

"calcium," "potassium," and "sodium" soils were prepared and used for a similar set of sorption measurements: they were made by the following method:

Thirty-gram lots of soil II were boiled up with two successive portions of *N* chloride solution (0.2 *N* for HCl), the supernatant solutions being decanted through Whatman filters.

After six washings with distilled water, boiling up and draining off each time, almost the whole of the soil samples had been washed on to the filters. The sodium soils were so sticky that drainage of the wash water was very slow and only three washings could be given before filtration practically ceased. The samples were then washed off the filters into their original conductivity beakers, boiled, cooled, and made up to weight by the usual method. The loss of soil in the process was negligible.

Conductivity results obtained with previously leached soils are shown in figure 5. Those for the calcium soil were practically identical with those for the original soil, which probably contained 80 per cent of its exchangeable base as calcium. Potassium and sodium soils gave an apparent sorption of calcium slightly greater than the original soil: they also gave a greater apparent sorption of hydrogen. Hydrogen soils gave decidedly lower (*B*-*A*) values and, at the same time, much greater increases in acidity than the original soils; e.g., for 0.01 *N* KCl an increase in C_H of 0.00055*N* was observed for the hydrogen soil in place of an increase of only 0.000002*N* for the untreated soil.

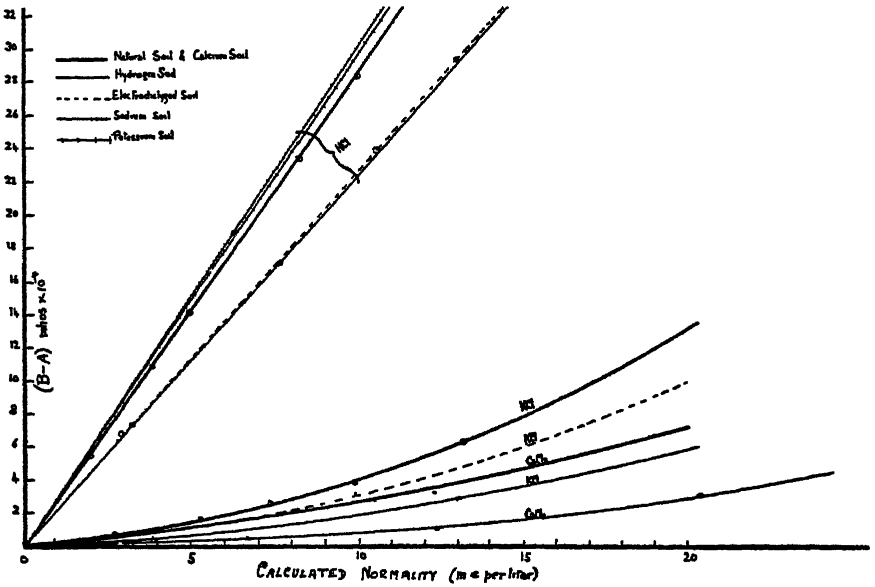

FIG. 5. CONDUCTIVITY RESULTS OBTAINED WITH LEACHED SOILS

As the sorption was thus affected by the cations originally present in the soil, only the electrodialyzed and "hydrogen" soils were used to compare hydrion replacements. In parallel with the conductivity measurements on these soils, a series of C_H titrations was carried out, first obtaining "blank" C_H curves for addition of the salts to water only. The extra increases in acidity of the suspensions were then used to give C values, or increases in conductivity due to hydrion exchange: these values are shown in figure 6.

For an increase in hydrion normality of x, the C value would be found by subtracting the specific conductivity of x N MCl from that of x N HCl.

The values then tabulated were: $(B - A + C)$ = calculated decrease in conductivity due to sorption: S = sorption in milligram equivalents per liter as read off from conductivity v concentration curves.

The S values for the "hydrogen" soil appear in figure 7 and show that, despite specific effects on the displacement of hydrogen, there is, in the case of potassium, calcium, and magnesium, an identical sorption—or total removal of a particular cation—from solution. The greater slopes of the curves for aluminum and hydrogen would be expected to result from the greater acidity of the solutions if, as it appears, there is any kind of ratio distribution between soil and solution.

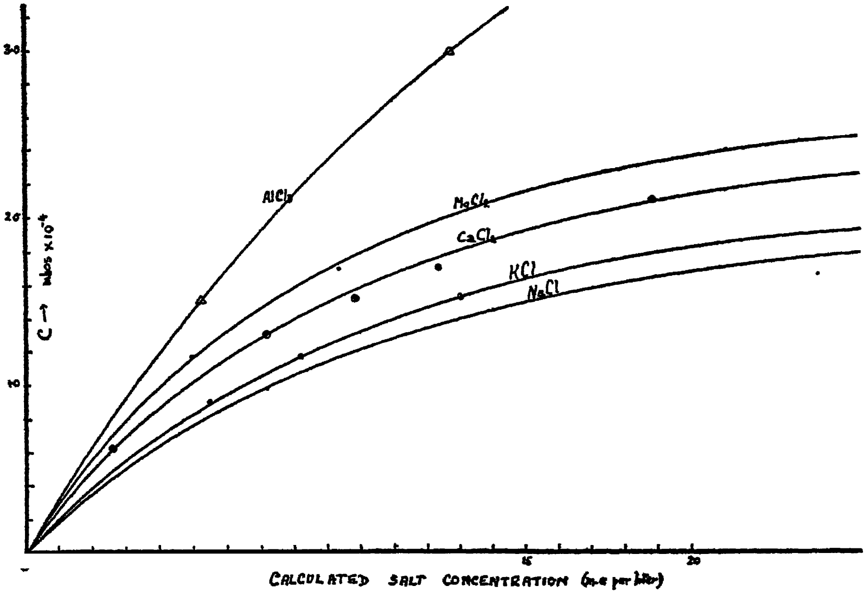

FIG. 6. INCREASES IN CONDUCTIVITY DUE TO HYDRION EXCHANGE

The curves for electrodialyzed samples of soil II showed sorption of hydrogen sodium, and potassium in equivalent amount and greater than that by the "hydrogen" soil. Now, the initial acidity of the suspensions of "hydrogen" soil, prepared by leaching soil II with hydrochloric acid, was greater than that of the electrodialyzed samples (pH about 3.3 instead of 3.8) and the hydrions in suspension would presumably compete in sorption with the metallic cations added. Thus the observed sorption would be less in the more acid solution. Such competition between cations has been noted by many investigators: for example, the calcium and potassium sorbed by hydrogels of silica and alumina have been shown to decrease with increasing hydrion concentration of the solution (4), whereas conductivity observations in the present series of experiments on addition of potassium chloride following oxalic acid to soil-suspensions show that, for the concentration range 0.002 N — 0.05 N, the hydrion sorbed decreases with increasing concentration of potassium ions.

The next step was to observe the effects on conductivity, pH and sorption values of previous heat-treatment of all the natural soils, and to compare these values with the others. As already described, the samples were heated to constant weight: the suspensions were given an extra weight of water equal to the loss of "combined water" on heating.

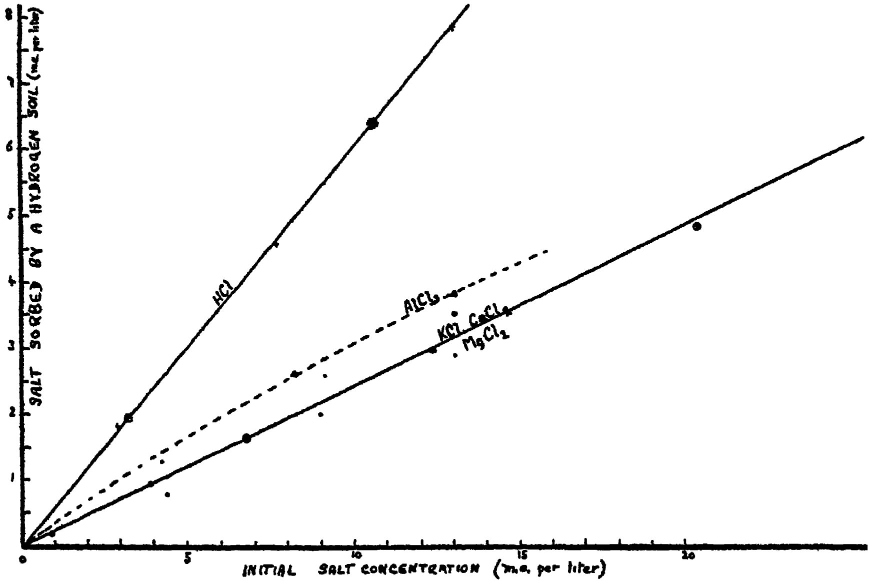

FIG. 7. SORPTION VALUES FOR THE "HYDROGEN" SOIL

TEMPERATURE EFFECTS

The effects of heating soils over various ranges of temperature are shown in table 4. A study was made of the resulting changes in specific conductivity, sorptive capacity as determined conductometrically, pH, and (T-S) values.

It is seen from the table that previous heating to 98°C. about trebles the specific conductivity of 1:5 aqueous suspensions: values for soils previously heated at 105–110°C. are greater than those for soils heated at 98°C., whereas heating at 150°C. increases the conductivity to an even greater extent. Ignition, except for the calcarious soils in which the enormous conductivity increases can be accounted for as due to formation of soluble oxides, decreases the conductivity to about half that for the unheated soils. The table shows a marked increase in acidity for suspensions of previously heated soils, indicating removal of hydrion from the colloidal complex of the soil.

It appears that, for each soil, there is an optimum temperature between the limits 110–150°C. at which the liberation of soluble salts from the complex is greatest: it is at this temperature also that the sorption capacity (*B*-*A* values) for added cations and salts is greatest. These results indicate some kind of heat-activation of the colloidal soil complex.

In order to see whether the sorption problem for soils could be attacked in a more direct manner, it was decided to seek similarity between the conditions already described and those obtaining for colloidal silicic acid. For this purpose, three silica hydrogels were prepared by stirring solutions of hydrochloric acid into an equivalent amount of 2 *N* sodium silicate solution: they were washed by decantation, filtered off, forced through a dye onto gauze frames, and dried in a current of air at room temperature. The hard, glassy granules so obtained were purified by electrodialysis and then dried to constant weight at room temperature.

TABLE 4

Temperature effects on soils

TREATMENT	pH	A	S	pH	A	S	pH	A	S	pH	A	S	pH	A	S
	Soil I			Soil II			Soil III			Soil IV			Soil V		
Boiled	4.6	1.40	2.17	8.2	2.81	3.33	8.5	1.03	1.98	7.9	1.96	....	9.0	0.70	1.39
18°C.	5.0	1.06	2.59	7.9	2.40	3.39	8.2	0.74	2.31	7.6	1.21	....	8.8	0.46	1.90
98°	....	3.75	....	....	10.45	....	....	4.18	2.72	....	3.62	....	....	4.94	....
108°	3.9	3.56	2.42	6.0	11.60	4.30	6.8	5.97	2.72	7.1	7.95	....	7.2	7.40	2.93
120°	3.4	6.31	2.93	4.6	16.10	....	5.6	....	2.83	....	.....	....	7.0	5.39	3.58
160°	....	5.80	3.09	....	14.00	4.20	....	8.15	2.40	....	10.16	....	....	3.87	2.71
Ignited	5.5	0.93	1.57	....	2.74	1.55	6.0	3.00	2.06	11.0	.....	....	....	40.70	....

A = specific conductivity of 1:5 aqueous suspension in mhos × 10^{-4}.

S = apparent sorption in mille equivalents per liter from 0.01 *N* KCl solution (neglecting corrections for changes in acidity).

TABLE 5

Properties of three gels

	APPROXIMATE COMPOSITION	H_2O	pH		SPECIFIC CONDUCTIVITY (10^{-4} MHOS)	
			1:5	1:50	1:5	1:50
		per cent				
Gel I	$SiO_2 \cdot H_2O$	23	....	4.20	...	0.20
Gel II	$SiO_2 \cdot 2H_2O$	37	3.72	....	1.1	0.15
Gel III	$SiO_2 \cdot 3H_2O$	47	3.74	4.64	1.6	0.22

The three gels employed had the properties shown in table 5.

The gels were graded by sieving, the granules used being 1 to 2 mm. in diameter. Conductivity measurements on suspensions of particles of diameter less than 0.2 mm. gave the same values only when allowed to stand for some time after stirring: the presence of such particles between the electrodes increased the electrical resistance of the suspensions. But, apart from this, particle size, and therefore surface area, had apparently no effect on the degree of sorption.

The conductivity and sorption values were unaffected by previous boiling.

Conductivity and pH titrations were made on aqueous gel-suspensions in a similar manner to those described for the soils; but the pH measurements had to be made on the "poured off" equilibrium solutions, as the presence of gel particles caused potential drift. It was therefore necessary to use a number of separate suspensions for each titration, withdrawing a fresh sample of solution for each pH measurement.

The three silica gels differed in the extent to which they liberated hydrions or sorbed cations according to their original composition. The gel prepared from the weakest acid (0.5 *N*) and having the highest water-content showed least hydrion exchange but greatest sorptive capacity.

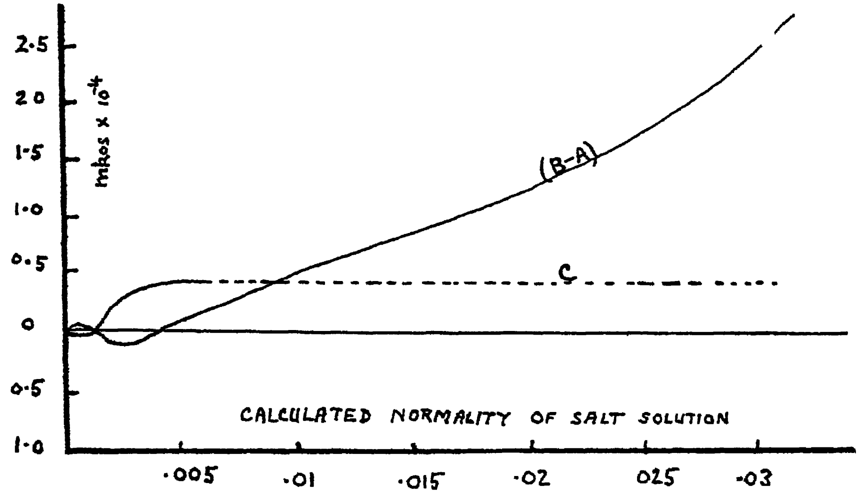

FIG. 8. (*B-A*) AND C CURVES FOR GELS

In addition to the usual chloride solutions, KNO$_3$ and K$_2$SO$_4$ were employed for conductivity and pH titrations, the concentration range being up to 0.05 *N*.

Attempts were also made to trace changes in chlorion concentration potentiometrically, using Ag: AgCl electrodes: they failed to yield concordant results. Such changes were estimated for 0.05 *N* solution by titrating the filtrates with 0.01 *N* AgNO$_3$, using di-chlor-fluorescein as indicator (6). Corresponding volumetric estimation of calcium, after precipitation as oxalate, showed removal of calcium and chlorine in equivalent amounts. But since the hydrion exchange or excess of cation to chlorion assumed to be removed from solution is only one-tenth of the total sorption in 0.01 *N* solution and also has greatest effect in the most dilute solutions, volumetric methods can hardly be relied on as a test of unequivalent sorption of anion and cation.

The gels gave greater exchange with hydrogen than the soils, the C values being about one tenth of the (B-A) values for 0.01 N solutions. The relatively greater extent of hydrion exchange resulted in more complicated forms of (B-A) and C curves, which, although differing in extent according to the particular gel or cation used, were of the general form shown in figure 8. Though exaggerated for gel III, the figure underestimates the case for gel I, the gel giving the greatest hydrion exchange.

Below salt-concentrations of 0.002 N, very slight decreases both in acidity and in conductivity were observed, showing that addition of very small proportions of metallic cations can cause an increase in the sorption of hydrion from solution. With increasing concentration of salt, however, there followed a definite slight increase in conductivity accompanied by considerable increase in acidity, sorption being masked by exchange, until, in concentrations between 0.005 N and 0.01 N, there was little further hydrion exchange but an increasing sorption of ions from solution.

The two curves shown were combined to give ($B - A + \mathrm{C}$) values, which were used to read off from the original "blank" curves the S values, or actual decreases in milligram equivalents per liter, of the particular cation employed. By this method, sorption concentration curves were obtained for all the salts and acids employed.

The only specific effects were those on hydrion exchange. The C_H curves varied in steepness according to the particular chloride on nitrate employed: displacement of hydrion was greatest in the order Ca > Mg > K > Na. The results for aluminum ions could not be compared with these because of the much higher proportion of hydrions present in aluminum chloride solution. Apart from the initial exchange reaction, no preferential order of cation sorption could be observed. The hydrions in solution must necessarily have a secondary effect on the sorption of any particular cation: the cation which replaces hydrogen to the greatest extent initially will be the most affected by competition with unlike ions.

The results obtained from conductivity and pH titrations on the 1:5 gel suspensions are given in table 6. Only the readings for gels II and III are included, partly for the sake of brevity and partly because the measurements for gel I were mostly taken on 1:50 suspensions instead of the 1:5 ratio employed in all the other cases. The 1:50 suspensions gave smaller sorption values with therefore a greater possible percentage error; otherwise there was no difference in the form of results obtained.

Table 6 shows that hydrion exchange in the gels has initially the greater effect on conductivity, whereas, with increasing concentration of salt, the predominating factor is removal of the salt as a whole. When the salt concentration has reached 0.01 N, most of the exchangeable hydrogen has been liberated: for potassium chloride, the C_H of gel II has increased from 0.00019 N to 0.00035 N, and that of gel III from 0.00018 N to 0.00025 N.

Although the final sorption curves resulted from a number of separate processes, they presented a remarkable uniformity. When sorption for any

TABLE 6

Addition of salts to 1:5 gel suspensions

N = normality of added salt in m.e. per liter.
A = observed specific conductivity in mhos × 10^{-4}.
B = calculated specific conductivity in mhos × 10^{-4}.
C = increase in specific conductivity due to hydrion exchange.
S = m.e. salt sorbed per liter (read off from conductivity graph).

	N	A	B	$B-A$	C	$B-A+C$	S	INCREASE IN C_H
								m.e./liter
Gel III								
NaCl	4.2	5.79	6.09	0.30	0.09	0.39	0.37	0.04
	11.6	12.74	13.48	0.74	0.16	0.90	0.85	....
	15.9	16.63	17.67	1.04	0.18	1.22	1.15	....
	21.5	21.61	23.00	1.39	0.19	1.58	1.50	....
	29.9	27.11	29.05	1.94	0.19	2.13	2.02	....
	38.6	36.29	39.25	2.96	0.20	3.16	3.00	....
	44.8	41.41	44.91	3.50	0.20	3.70	3.55	0.07
KCl	5.5	8.16	8.48	0.32	0.13	0.45	0.35	0.04
	8.5	11.53	12.16	0.63	0.14	0.77	0.60	....
	11.3	14.71	15.59	0.88	0.15	1.03	0.81	....
	14.2	17.90	19.02	1.12	0.15	1.27	1.00	....
	18.8	22.88	24.27	1.39	0.16	1.55	1.22	....
	23.6	28.00	29.95	1.95	0.20	2.15	1.70	....
	26.8	31.38	33.58	2.20	0.21	2.41	1.91	....
	33.1	37.82	40.75	2.93	0.22	3.15	2.51	0.08
$MgCl_2$	2.2	3.81	3.81	....	0.12	0.12	0.11	0.04
	6.3	7.56	7.83	0.27	0.16	0.43	0.41	....
	8.8	9.77	10.26	0.49	0.17	0.66	0.62	....
	14.9	14.91	15.79	0.88	0.19	1.07	1.01	....
	20.4	19.62	20.75	1.13	0.20	1.33	1.27	....
	38.0	33.38	35.60	2.22	0.22	2.44	2.34	....
	46.5	23.65	42.72	2.90	0.23	3.13	2.98	0.07
$CaCl_2$	3.4	5.20	5.33	0.13	0.14	0.27	0.25	0.04
	6.1	7.82	8.12	0.40	0.15	0.55	0.49	....
	9.3	10.82	11.33	0.51	0.17	0.68	0.61	....
	12.2	13.40	14.13	0.73	0.17	0.90	0.81	....
	16.6	17.18	18.30	1.12	0.18	1.30	1.18	....
	36.6	34.11	36.50	2.39	0.20	2.59	2.36	....
	47.7	42.79	46.25	3.46	0.20	3.66	3.33	0.06
$AlCl_3$	9.1	15.28	11.49	−3.79	5.00	1.21	0.95	2.03
	13.2	19.71	15.80	−3.91	5.91	2.00	1.59	....
	18.2	25.23	21.00	−4.23	6.78	2.55	2.02	....
	25.6	32.24	28.45	−3.79	7.70	3.21	2.56	....

TABLE 6—*Continued*

	N	*A*	*B*	*B* − *A*	*C*	*B* − *A* + *C*	*S*	INCREASE IN C_H m.e./liter
Gel III—Concluded								
KNO_3	3.7	5.90	6.22	0.32	0.10	0.42	0.33	0.04
	8.4	11.03	11.66	0.63	0.14	0.77	0.65	....
	11.5	14.29	15.14	0.85	0.15	1.00	0.84	....
	17.4	20.54	21.74	1.20	0.16	1.36	1.14	....
	22.3	25.35	27.22	1.87	0.18	2.05	1.67	....
	30.8	33.87	36.29	2.42	0.21	2.63	2.16	....
	41.2	43.62	47.04	3.42	0.22	3.64	2.95	0.08
K_2SO_4	6.1	8.41	8.85	0.44	0.10	0.54	0.42	0.04
	8.9	11.30	12.07	0.77	0.12	0.89	0.71	....
	12.5	14.89	15.88	1.01	0.15	1.16	0.91	....
	33.0	34.11	36.75	2.64	0.17	2.81	2.27	0.08
HCl	2.7	10.54	11.65	1.11	....	....	0.30	....
	4.4	16.03	17.91	1.88	....	....	0.50	....
	6.9	24.17	27.08	2.91	....	....	0.78	....
	10.7	36.78	41.44	4.66	....	....	1.24	....
	15.1	50.60	57.76	7.16	....	....	1.90	....
	21.8	71.06	83.02	11.96	....	....	3.19	....
	27.4	88.41	104.41	16.00	....	....	4.26	....
	33.6	105.64	128.36	22.72	....	....	6.45	....
	42.3	132.10	162.44	30.34	....	....	8.08	....
Gel II								
NaCl	3.5	4.88	4.81	−0.07	0.28	0.21	0.20	0.11
	6.8	8.09	8.09	....	0.35	0.35	0.33	....
	12.2	13.21	13.45	0.24	0.40	0.64	0.60	....
	16.8	17.40	17.91	0.51	0.43	0.94	0.89	....
	24.5	24.36	25.18	0.82	0.45	1.27	1.20	....
	52.9	48.86	51.43	2.57	0.47	3.04	2.87	0.18
KCl	2.2	4.06	3.94	−0.12	0.27	0.15	0.12	0.11
	4.6	6.97	7.00	0.03	0.33	0.36	0.28	....
	10.9	14.29	14.64	0.35	0.38	0.73	0.57	....
	16.2	20.13	20.78	0.65	0.39	1.04	0.82	....
	23.4	28.00	29.16	1.16	0.40	1.56	1.18	0.18
$MgCl_2$	4.6	5.86	5.71	−0.15	0.37	0.22	0.21	0.18
	13.0	13.40	13.53	0.13	0.49	0.62	0.58	....
	18.8	18.32	18.64	0.32	0.52	0.84	0.79	....
	31.7	28.55	29.59	1.04	0.60	1.64	1.57	....
	37.1	33.24	34.38	1.14	0.60	1.74	1.66	....
	47.3	41.14	42.98	1.84	0.60	2.44	2.35	0.24

TABLE 6—*Concluded*

	N	A	B	$B-A$	C	$B-A+C$	S	INCREASE IN C_H
								m.e./liter
				Gel II—Concluded				
$CaCl_2$	4.3	5.83	5.71	−0.12	0.45	0.33	0 30	0.18
	8.2	9.77	9.68	−0.09	0.53	0.44	0.40	....
	12.2	13.59	13.64	0.05	0.58	0.63	0.57	....
	18.5	19.41	19.63	0.22	0.62	0.84	0.76	....
	23.5	23.88	24.17	0.29	0.63	0.92	0.87	....
	40.4	38.52	39.35	0.83	0.63	1.46	1.32	0.24
$AlCl_3$	2.2	6.30	4.16	−2.14	2.75	0.61	0.47	....
	16.5	23.56	20.19	−3.37	6.11	2.74	2.18	....
	22.8	29.73	26.57	−3.16	6.90	3.74	3.06	....
	34.6	39.08	37.99	−1.09	8.16	7.07	5.70	....
HCl	1.5	5.98	6.53	0.55	....	....	0.15	....
	2.7	9.98	11.08	1.10	....	....	0.29	....
	5.7	20.25	22.05	1.80	....	....	0.48	....
	8.2	28.34	31.10	2.76	....	....	0.74	....
	12.8	43.91	48.70	4.79	....	....	1.28	....
	20.5	69.18	77.48	8.30	....	....	2.21	....
	24.7	83.26	93.62	10.36	....	....	2.76	....

particular gel was plotted against initial concentration of added salt, *straight line graphs resulted which, with the exception of the acids and aluminum salts, were identical for all the salts employed.* These results are shown in figure 9, in which the plotted points are all for gel III—the gel of least hydrion exchange but greatest sorptive capacity—while corresponding sorptions for gel II are shown by the dotted lines.

The results indicate equivalent sorption of Na, K, Mg and Ca. The coincidence of the graphs obtained for KCl, KNO_3, and K_2SO_4 indicates absence of anion influence. The greater sorption of Al and H by both soils and gels may be due to the greater acidity of the solutions. For the case of soils, where hydrion exchange was slight, there might possibly be an exchange with the aluminum of the soil-complex to as great an extent as that with hydrogen: the greater sorption of Al would then be in accordance with the partition coefficient distribution.

Addition of an aqueous solution of KOH to suspensions of soils and of gels caused rapid fall in conductivity to equilibrium values, indicating neutralization reactions or else comparatively great sorption. Whether hydroxl ions are only neutralized at the surface of the soil particles or are definitely adsorbed as stated by Mattson (7) and others, is questionable. Recalling the results of electrodialysis of soils, the author found the anions estimated by titration with alkali to be only about one-eighth of the total cations, no silicate ions being detected in the anode chamber. Now, if hydroxlyion from the soil complex took part in conducting the current through the cells, it could not be detected as "adsorbed" hydroxyl ion since this ion is also liberated by the current in the cathode compartment.

Colloidal silica is said to have a negative charge in solution; and it seems possible that OH^- ions and replacing Cl^- ions are concentrated at the surface of the particles in accordance with some kind of membrane equilibrium which, however, is undisturbed by mechanical shaking.

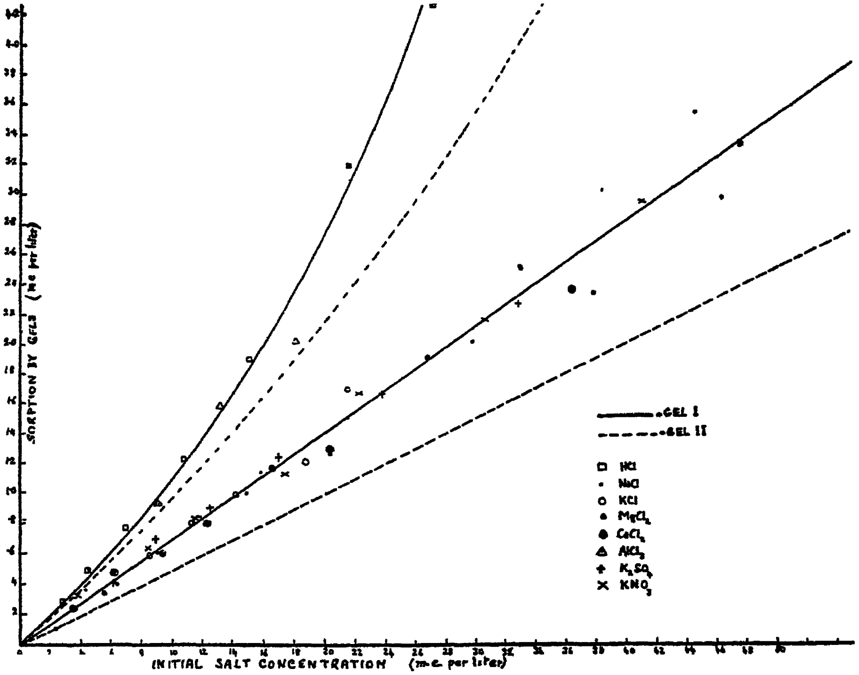

FIG. 9. SORPTION FOR GELS I AND II PLOTTED AGAINST INITIAL CONCENTRATION OF ADDED SALT

The effect of previous heating on the gels was quite different from that on soils. Samples of gel which had lost all their "combined" water on ignition gave out 30 heat calories per gram on wetting with water: after drying they had exactly regained their original water content and gave identical sorption values.

The analogy between soils and gels (2), however, must not be driven too far: comparison of temperature effects shows the soil complex to have a much more complicated structure.

DISCUSSION OF THE RESULTS

If the empirical equation of Freundlich holds, values of log a/log c, (where a = amount sorbed per unit weight absorbent and c = equilibrium concentration of solution) should give a straight line graph. For the sorptions here investigated and corrected for changes in acidity, this is the case.

If sorption of electrolytes is to be compared with that of non-electrolytes it must, according to Gibbs' condensation theory, correspond with the separate ionic effects on the interfacial tension and result from sorption of both ions individually. Adsorption of salts from solution has been studied chiefly with charcoals for which anion and cation are adsorbed to an equivalent extent, displacing ions of like sign in the order of their adsorbability which, for cations, is H > Al > Ca, Mg > NH_4 > K and Na: acids and bases are adsorbed to a greater extent than their salts (11). Although sorption by charcoals is always equivalent, silicic acid and powdered iron oxide may adsorb anion and cation to a markedly different extent (9). Since accumulation of charge in the solution is impossible it is assumed on the exchange theory that the adsorbed ion is replaced by some other mobile ion liberated from the complex, and that unequivalent adsorption occurs only when a mobile ion is present in the sorbent.

In the present experiments on silica gel suspensions, a considerable increase in acidity was found invariably to result from addition of neutral salts between the ranges 0.002–0.01 *N* indicating an exchange reaction of the type $K^{\cdot} + Cl^{-} + H_2SiO_3 \rightarrow H^{\cdot} + Cl^{-} + K_2SiO_3$. But pH titrations showed this exchange to be greatest initially and to be very slight after a salt concentration of 0.01 *N* had been reached. Corresponding conductivity measurements showed an increased sorption of the added cation after all exchangeable hydrion had been replaced.

For concentrations of chloride below 0.002 *N*, a slight increase in alkalinity was sometimes observed: it appears, therefore, that very small proportions of metallic cations can cause an increase in the sorption of hydrion from solution. This explains the initial bend in the (*B-A*) curves which corresponds with that of the C curves, as shown in figure 8.

If the order of "sorbability" of the cations in silica gels is the same as that of their exchange with hydrogen, it is H > Al > Mg and Ca > K > Na which is the familiar order of valency, hydrion excepted. On the other hand, work on both gels and electrodialyzed, or "hydrogen," soils showed equivalent sorption of Na, K, Mg, and Ca, the word *sorption* meaning the *total* removal of cation both by exchange and by ab- or adsorption. The greater sorption of Al and H might simply be due to the greater acidity of the solutions.

The process involving removal of anions and cations from solution by soils and by silica gels is thus of a dual nature: the initial reaction is chiefly one of exchange but this is smaller in extent than the second process, which is probably removal of the salt as a whole.

SUMMARY

A study has been made of conductivity and pH measurements on aqueous suspensions of soils and silica gels, correlating the results with those obtained for samples which had been subjected to an initial heat-treatment.

Addition of neutral chloride solutions increases the acidity of soil and gel suspensions but effects a simultaneous decrease in conductivity. This shows sorption of ions in great excess to those taking part in "base exchange."

In the sorption reactions studied, there is immediate attainment of equilibrium.

Different cations are shown to displace hydrogen preferentially; but experiments on a "hydrogen" soil and on silica hydrogel show equivalent sorption of the cations Na, K, Mg, and Ca.

For five typical soils, an optimum temperature, between the limits 110°–150°C., was found to give maximum values for the acidity and conductivity of resulting suspensions and also for subsequent sorption of cations. For silica hydrogels, however, the effects appear to be completely reversible.

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AN IMPROVED METHOD OF MEASURING SOIL COLOR

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The method of measuring the color of a soil by comparison with a rotating disc composed of segments of black, white, red, and yellow paper was developed by Hutton and Rice of the Color Standards Committee of the American Soil Survey Association (1, 2). When this method is used the exposed portions of the four paper discs are altered until the blending of these when rapidly rotated gives a color comparable to that of the soil, which is held in a small dish or on a broad bladed spatula either over the rotating surface or closely beside it. Soils are granular, and even when they are carefully smoothed the diffusion of light reflected from the surface of the many particles, together with the shadows in the recesses of the soil, gives color values that are very hard to match.

A method of preparing and mounting the soil sample has been devised that permits of rotating the soil as the central part of the disc area thereby getting a mixed or composite light effect and color value that is very similar to that of the rotating color disc and which permits a much easier matching of the colors.

The soil is made into a suspension in about three times its volume of water, and is mixed until well dispersed. This mixing can be done by rubbing with the fingers or a rubber pestle or by boiling and pestling to destroy the aggregates. Usually it is well to let the soil stand for 12 to 24 hours after dispersing. The soil "mud" is then painted onto white blotting paper, using a small brush, and making sure that the blotting paper is so well coated that it does not show through the soil. When the soil-coated paper is thoroughly dry a disc 2 or 2½ inches in diameter is cut out, a hole punched in the center, and this disc mounted on the face of the color disc and fastened down by the lock nut (plate 1, fig. 1). It is well to use a washer of mounted blotting paper between the soil disc and the lock nut to prevent chipping and cracking the soil surface. When rotated the soil disc develops a smooth color effect similar to that of the color discs and a good match in color values is readily obtained (plate 1, fig. 2), As the soil disc is mounted on the inner circle of the disc assembly, comparison of the color effects is easy.

Nearly all soils will adhere to the blotting paper and bear considerable handling without the coating rubbing thin. Sandy soils are most difficult in this respect, although some of the heaviest clays have a tendency to crack and the shadows in the cracks tend to give a darker shade. The color of the soil obtained by this method is essentially that of a dried and crusted soil or the surface of a dried clod, and may not truly represent the color effect of the granulated or broken soil surface as seen in the field. It is a natural color effect, however, and can be compared with other soils in the same condition. It can be readily reproduced with consistent measurement results, and affords a means of definitely measuring and comparing the color effects of soils.

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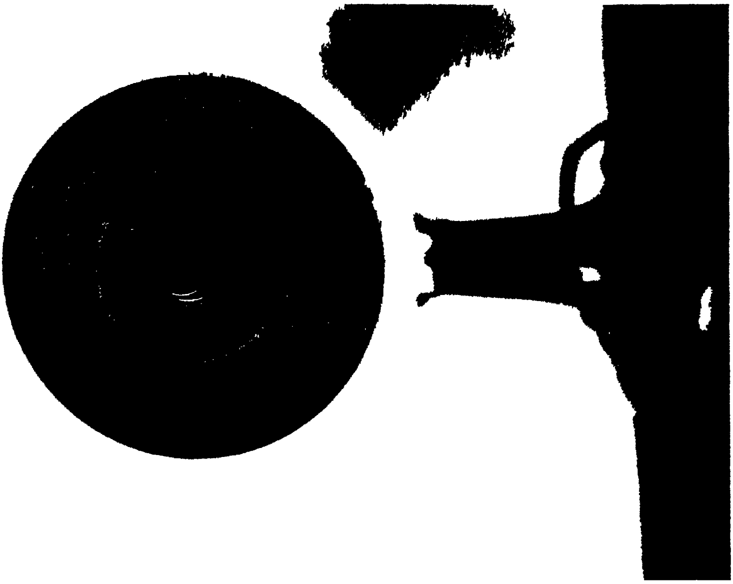
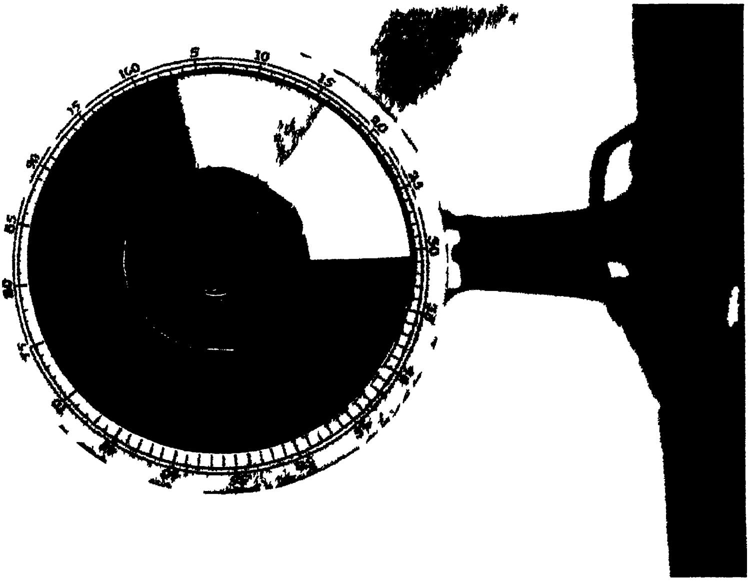
PLATE 1

The Soil Colorimeter

Fig. 1. Colorimeter at rest with soil disc mounted in place. Exposed segments show black 69½, white14½, yellow 13, red 3.

Fig. 2. Colorimeter rotating. Match of color values is not exact, the soil disc showing slightly darker. Final adjustment gave black 71, white 14, yellow 12, red 3.

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FACTORS AFFECTING THE AMOUNT OF ELECTRODIALYZABLE IONS LIBERATED FROM SOME SOILS

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In an earlier paper (10) the author gave a description of the method and apparatus used in liberating the exchangeable cations from soils by electrodialysis. As the principle of electrodialysis has been adapted more and more into the field of soil investigations, a study of the factors which influence the ion exchange process is desirable. Of special interest is an investigation of the conditions affecting the speed of the replacement of ions which takes place in the soil suspension.

The author believes that the various conditions influencing the amount of ions given off from soils by electrodialysis should be accurately controlled in order to get reproduceable results. Then the results would give more information about the problem studied, and the data presented of various investigators should be much more comparable.

An important investigation as to the apparatus and technique of electrodialysis applied to the study of soils has just been reported by Odén and Wijkström (13). Of the data presented in this paper the most interesting in this connection are those which deal with the influence of temperature on the speed of the liberation of ions. The authors have found that increases in temperature cause only a slight increase in the quantity of exchangeable cations liberated, whereas the weathering effect on the soil particles is greatly increased. The influence of the temperature differs for different soils and for the various cations; the numbers given show that the increases in cation liberation per degree Celcius vary from 0.02 to 2.5 per cent for the replaceable cations and from 0.7 to 11.0 per cent for the quantities of the liberated cations which are supposed to descend from weathering effect or breaking down of the soil particles.

This paper deals with the study of the amounts of ions liberated and the speed of ion liberation from certain soils in relation to the concentration of the soil suspension used, to the distance between the electrodes, to the effect of increased voltages, and also to the influence of different membranes. These factors vary frequently in different experiments and it is believed that data which illustrate the influence of the mentioned factors will be of interest.

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[1] The present investigation was made by the writer while at Cornell University and Ohio State University as a Fellow of the Rockefeller Foundation.

Comparison between the amounts of replaceable cations found by electrodialysis under various conditions and the amounts found by extraction with a neutral salt solution is also made.

SOILS AND METHODS USED IN THE INVESTIGATION

Six soils were used in the investigation; three of these belong to the Volusia silt loam type and three to the Ontario loam type. The chemical composition of these soils has been determined by Bizzell (3), and his analytical data are given in table 1.

The numbers in table 1 refer to the total amounts of the elements studied calculated in percentage of dry soil. The samples were collected to a depth of 8 inches, had passed through a sieve containing circular perforations 1 mm. in diameter, and then were pulverized in an agate mortar to a very fine powder (100 meshes to 1 linear inch) before the analytical procedure took place.

TABLE 1

Chemical analysis of the soils used in the investigation

(After Bizzell)

LABORATORY NUMBER	SOIL TYPES	CONSTITUENTS IN SOIL, DRY BASIS					
		Nitrogen	Phosphorus	Sulfur	Potassium	Calcium	Magnesium
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
7A	Volusia silt loam	0.194	0.076	0.064	1.66	0.18	0.22
49A	Volusia silt loam	0.134	0.046	0.063	1.81	0.49	0.52
50A	Volusia silt loam	0.205	0.078	0.071	2.17	0.41	0.64
14A	Ontario loam	0.143	0.048	0.063	2.44	1.08	0.85
48A	Ontario loam.	0.125	0.052	0.037	1.64	0.56	0.51
79A	Ontario loam	0.404	0.119	0.057	1.98	0.84	0.73

The mechanical composition of the mineral fraction in the samples was determined by the method of Atterberg (1) and the results are recorded in table 2. The table also gives the content of organic material as determined by the hydrogen peroxide method (14), the water content in air-dry soils, and the pH values determined by the quinhydrone electrode.

The samples used in the author's experiments were passed through a sieve with openings 1 mm. in diameter and the data given in table 2 and following tables have been calculated on dry-weight basis.

The experiments with Volusia silt loam were made with Bradfield's (6) modification of the three-compartment electrodialysis cell and the experiments with Ontario loam were, with one exception, made with the cell devised by the author (10). The conditions under which the different experiments were performed have been varied more or less as to the problem to be studied. Such changes will be noted separately in connection with the description of the experiments. In this connection it should be mentioned that all experiments were supplied with effective stirring of the soil suspension, and that the temperature, unless it is especially mentioned, was controlled and held constant at 23–25°C. without incorporation of resistance in the electrical circuit. By such an arrangement the voltage between the electrodes is kept constant during the time of experimentation. Concerning the temperature control it should be stated that in the experiment with the Volusia silt loam soils no cooling system was required in order to hold the aforementioned temperature limits because these soils are poor in replaceable cations. In the experiments where the Ontario loam soils were used, however, the temperature in the middle chamber, without a cooling system applied, arose within a short time to about 40–50°C. By a cooling arrangement as described by the author (10) the temperature was easily held within the desired range by ordinary tap water.

In plate 1, fig. 1, is shown the author's electrodialysis apparatus.[2] Several kinds of stirring arrangements and stirring motors have been tried. A stirring motor which especially can be recommended is the type recorded as C. L. 2 from Bodine Electric Co., Chicago. Ammeter, voltmeter, and a knife switch have been incorporated in the electrical circuit.

TABLE 2

Mechanical analysis, organic material, water content, and pH values of the soils used in the investigation

SOIL NUMBER	SOIL FRACTION <1.0 MM. (DRY BASIS)					WATER CONTENT	pH
	Mineral material				Organic material		
	1.0–0.2 mm.	0.2–0.02 mm.	0.02–0.002 mm.	<0.002 mm.			
	per cent	*per cent*	*per cent*	*per cent*			
7A	14.08	47.22	19.19	16.61	2.90	1.46	5.35
49A	16.26	55.40	16.35	9.67	2.32	1.43	5.60
50A	6.88	48.80	24.25	16.47	3.60	2.10	5.25
14A	14.68	37.80	28.75	16.20	2.57	1.87	7.50
48A	16.75	46.03	27.35	7.62	2.25	1.27	6.25
79A	9.59	50.43	25.10	6.88	8.00	2.83	7.40

The experiments have been performed as fractional electrodialysis. The procedure of such experiments is briefly: The dialysates in the end chambers are removed at certain intervals and the chambers are refilled with distilled water, while the fractions obtained can be used for determination of the ions liberated. At the same time as the end chambers are drained a small portion of the soil suspension can be removed from the middle chamber for various measurements. After the desired measurements of the soil portions are taken, they must be dried and weighed in order that the amount of soil remaining in the middle chamber can be calculated.

In most of the experiments which are reported here the length of the intervals between each changing of the dialyzates have been 12 hours. A more frequent changing of the dialyzates would, according to Odén and Wijkström (13) facilitate the exchange process. The purpose of these experiments, however, has not been to remove the exchangeable cations as soon as possible in these particular cases, but to study the conditions which influence the speed of the reactions which take place.

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[2] The apparatus can be supplied by the Central Scientific Company of Chicago.

The fractions from the end chambers were poured into 200-cc. volumetric flasks, filled to volume, and portions of 50 cc. were used for determinations of the total amounts of cations and anions liberated in the different intervals. The determinations were made by titration against HCl and NaOH, using methyl red and phenolphthalein as indicators respectively. The normality of the reagents used has been 0.1 N and 0.01 N.

Besides the determination of the amounts of electrodialyzable cations liberated under various conditions, an investigation concerning the amounts of replaceable cations determined by help of extraction with ammonium acetate was made. By reason of its neutral character and its significant buffer capacity around the neutral point, this salt should be very favorable for use in base exchange work because the solubility effect on the soil particles should be very small. Also for other reasons this salt has advantages, one of the most important for the following analytical procedure of the extracts might be the ease by which the ammonium salt can be expelled [*see* Schollenberger (15) and Schollenberger and Dreibelbis (16)].

The method of mechanical dispersion and extraction used has, with some modifications, been that described by Bouyoucos (4) and Baver (2). Twenty-five grams of fine soil ($<$ 1.0 mm.) is placed in a 250-cc. beaker, then 100 cc. of normal $NH_4C_2H_3O_2$ solution is added and the suspension is stirred for 10 minutes by a motor driven stirrer (plate 1, fig. 2a). The sample is then poured over into a Buchner funnel and, according to Baver, leached with a new 100-cc. portion of the ammonium acetate solution. Suction is applied in order to facilitate the leaching process.

In order to get a uniform amount of filtrate from all soils the author has changed the leaching procedure of the method, and instead of washing the sample in the funnel with a new portion of 100 cc. acetate solution the filtration is continued till 200 cc. of filtrate is obtained. For this purpose a combined suction-volumetric flask, which is shown in plate 1, fig. 2b, has been made. Filtration by help of this type of flask has proved to go very rapidly in relation to that with the Witt's filter apparatus or with the filter flask of Erlenmeyer form.

The solubility effect on the soil particles due to leaching with ammonium acetate solution has been studied by continued washing of the original samples until, in all, four fractions of 200 cc. each were obtained from each of the six soils investigated. The content of water-soluble salts in the soils was also determined. In this case the samples were treated in the same way as with the ammonium acetate method, but instead of salt solution distilled water was used as the solution agent. The amounts of exchangeable cations and water-soluble salts in the soils were, after evaporation of the filtrate with concentrated HCl and expelling of the ammonium salt, determined as total chlorides first by weight and then by titration of the chloride ions with 0.1 or 0.01 *N* $AgNO_3$ solution using potassium chromate as indicator. As a control, aliquots of the filtrates have been electrodialyzed and the amounts of cations liberated by the leaching process were determined by titration of the cathode water by 0.1 *N* HCl solution using methyl red as indicator.

INFLUENCE OF VARIOUS FACTORS ON THE RATE AND AMOUNT OF ION LIBERATION FROM SOILS

Difference in the concentration of the soil suspension

In fractional electrodialysis experiments the concentration of the soil suspension decreases from fraction to fraction. Thus it will be understood that it is necessary to start with a large soil sample in order to get sufficient amounts of ions for the analytical procedure in the last fractions. The results obtained for each of the various fractions and for the whole experiment will then be more significant.

The extent to which difference in concentration of the soil suspension affects the rate of replacement of ions has received but little attention. The subject should preferably be studied under conditions where the concentration remains constant during the experiment, and the problem investigated by using different sized samples suspended in a given volume of water. In tables 3 and 4 are presented a series of results dealing with the aforementioned problem.

These tables contain results of experiments with soils 49A and 50A, and involve a comparison of 25-gm. and 50-gm. suspended in a bulb of 110 cc. volume. The distance between the electrodes was 6 cm., the voltage 110, and parchment was used as membranes.

Columns 3 and 7 in the tables show the amounts of cations, calculated in milliequivalents (M.e.) per 100 grams of soil, liberated from 25-gm. and 50-gm. samples, respectively. It is seen that in the first fraction a greater amount of cations is given off per unit weight of soils in the case of 25-gm. samples than in the 50 gm. samples. In the following fractions, the opposite is the case because more cations are left per unit weight in the 50-gm. sample. In the end, however, the total amounts of cations liberated are about the same in the two experiments.

The amount of cations given off per hour is given in columns 4 and 8. As we see, the amounts drop down to a minimum below 0.010 M.e. in the latter fractions. In the experiment with soil 49A this low value is reached after 36 hours when a 25-gm. sample was used but 60 hours was required to reach this same value in the experiment with 50 gm. In the experiment with soil 50A, a similar tendency is seen; the low end value, however, is reached after longer periods of time, presumably because this soil is somewhat richer in exchangeable cations, and may also have a stronger adsorbing capacity due to a greater content of colloids and organic matter (table 2).

TABLE 3

Cations and anions removed from soil suspensions of varying concentration in various periods of time—soil 49A

FRACTIONS	DIALYZATES CHANGED AFTER	EXPERIMENT WITH 25-GM. SAMPLE				EXPERIMENT WITH 50-GM. SAMPLE			
		M.e. cations per 100 gm. of soil		M e. anions per 100 gm. of soil		M.e. cations per 100 gm. of soil		M.e. anions per 100 gm. of soil	
		Per fraction	Average per hour	Per fraction	Average per hour	Per fraction	Average per hour	Per fraction	Average per hour
1*	2	3	4	5	6	7	8	9	10
	hours								
a	12	2.474	0.206	0.538	0.030	1.689	0.141	0.342	0.029
b	24	0.444	0.037	0.234	0.020	0.875	0.073	0.165	0.014
c	36	0.206	0.017	0.140	0.012	0.347	0.029	0.159	0.013
d	48	0.085	0.007	0.109	0.009	0.198	0.017	0.100	0.008
e	60	0.069	0.006	0.084	0.007	0.161	0.013	0.104	0.009
f	72	0.057	0.005	0.083	0.007	0.090	0.008	0.104	0.009
g	84	0.057	0.005	0.093	0.008	0.092	0.008	0.097	0.008
h	100	0.073	0.005	0.118	0.007	0.093	0.006	0.099	0.006
i	125	.....	.....	... .	.....	0.148	0.006	0.163	0.007
Total..........		3.465	.....	1.219	.....	3.693	.....	1.333	.....

* Column no.

TABLE 4

Cations and anions removed from soil suspensions of varying concentration in various periods of time—soil 50A

FRACTIONS	DIALYZATES CHANGED AFTER	EXPERIMENT WITH 25-GM. SAMPLE				EXPERIMENT WITH 50-GM. SAMPLE			
		M.e. cations per 100 gm. of soil		M.e. anions per 100 gm. of soil		M.e. cations per 100 gm. of soil		M.e. anions per 100 gm. of soil	
		Per fraction	Average per hour	Per fraction	Average per hour	Per fraction	Average per hour	Per fraction	Average per hour
1	2	3	4	5	6	7	8	9	10
	hours								
a	12	2.926	0.244	0.730	0.061	2.239	0.187	0.609	0.051
b	24	0.707	0.059	0.243	0.020	0.980	0.082	0.176	0.015
c	36	0.324	0.027	0.114	0.010	0.483	0.040	0.091	0.008
d	48	0.247	0.021	0.108	0.009	0.265	0.022	0.073	0.006
e	60	0.133	0.011	0.099	0.008	0.219	0.018	0.065	0.005
f	72	0.124	0.010	0.091	0.008	0.112	0.009	0.062	0.005
g	84	0.101	0.008	0.085	0.007	0.086	0.007	0.057	0.005
h	100	0.088	0.006	0.104	0.007	0.083	0.005	0.081	0.005
i	125	.....	.....	.....	.....	0.122	0.005	0.125	0.005
j	150	.....	.....	.....	.....	0.112	0.005	0.122	0.005
Total.:........		4.650	.....	1.574	.....	4.701	.....	1.461	.....

Concerning the liberation of anions it will be seen in the tables that the amounts given off are much less than the amounts of cations, and also that the size of the samples has but little effect on the quantities found in the different fractions.

Experiments with soil 79A comparing 50-gm and 100-gm. of soil suspended in a bulb of 550 cc. volume were also performed. For the sake of brevity the data are not given, but it should be mentioned that the results were similar to those already described.

The advantage of using small soil samples is clearly indicated from the experiments. On the other hand the size of the samples should not be reduced too much, because of heterogeneity of the soil, which might cause an increase in the analytical error. For ordinary analytical purposes 25 gm. of mineral soil and 10 gm. of muck soil should be recommended.

Difference in the distance between the electrodes

Three series of experiments are performed in order to illustrate the influence of differences in the distance between the electrodes on the rate of ion liberation. These experiments, however, are comparable only to a certain extent because an increase in the distance between the electrodes results in an increase of the volume of the bulb in which the soil sample is to be suspended. In other words two of the experimental conditions are changed at the same time, and these might have more or less opposite effects. It was assumed, however, that an increase in the electrode distance followed by increasing resistance in the apparatus would dominate over the decrease in resistance which may occur as a consequence of the dilution of the soil suspension.

The experimental conditions were the following: Samples of 50 gm. of soil 79A were suspended into bulbs of 110 cc., 450 cc., and 550 cc. volume; the corresponding electrode distances were 6 cm., 9 cm., and 12 cm., respectively. The voltage in all experiments was 110, consequently the differences in potential between the electrodes expressed in volts per cm. were 18.3 volt/cm., 12.2 volt/cm., and 9.2 volt/cm. in the different cases. The membranes consisted of parchment at the cathode and cellophane at the anode. It should be stated that the temperature regulation was not entirely effective during the first interval in the experiment with 6 cm. electrode distance (for a short time it arose to about 50°C.), and the corresponding fractions of anode and cathode water (the a-fractions) might show too large amounts of ions because a rise in temperature facilitates the liberation of ions [Odén and Wijkström, (13)]. However, in the remainder of the experiments, and in the experiments with 9 cm. and 12 cm. electrode distance the temperature was held within the required limits of 23–25°C.

The results concerning the replacement of cations are recorded in table 5. Columns 3, 6, and 9 give the total amounts in the different fractions, and columns 4, 7, and 10, the average amounts per hour per fraction. In addition, the amounts of cations liberated per volt/cm. are given in columns 5, 8, and 11.

In table 6 the corresponding data concerning the liberation of anions are presented.

As seen from the tables, increases in the electrode distance from 6 cm. to 9 cm., and from 9 cm. to 12 cm. are followed by significant decreases in the amounts of ions removed from the soil. These decreases are especially visible in the first fractions. It will be understood that a comparison can be made with accuracy only between the first fractions, because the amounts of ions remaining in the soil suspension at the beginning of the later intervals have changed according to the different amounts removed during the first period. Therefore the later fractions are more or less incomparable, and only when this

TABLE 5

Influence of differences in the distance between the electrodes on the replacement of cations

FRACTIONS	DIALYZATES CHANGED AFTER	ELECTRODE DISTANCE 6 CM.			ELECTRODE DISTANCE 9 CM.			ELECTRODE DISTANCE 12 CM.		
		M.e. per 100 gm. of soil			M.e. per 100 gm. of soil			M.e. per 100 gm. of soil		
		Per fraction	Per hour	Per volt/cm.	Per fraction	Per hour	Per volt/cm.	Per fraction	Per hour	Per volt/cm.
1	2	3	4	5	6	7	8	9	10	11
	hours									
a	12	6.720	0.560	0.367	3.680	0.307	0.302	2.480	0.207	0.270
b	24	2.720	0.227	0.149	2.560	0.213	0.210	1.860	0.155	0.202
c	36	1.836	0.153	0.100	1.786	0.137	0.146	1.600	0.133	0.174
d	48	1.469	0.122	0.080	1.380	0.126	0.113	1.240	0.103	0.135
e	60	1.195	0.100	0.065	1.360	0.113	0.111	1.000	0.083	0.109
f	72	1.236	0.103	0.067	1.153	0.096	0.095	0.840	0.070	0.091
Total...		15.176	.....	.....	11.919	.....	.....	9.020	.....	.....

TABLE 6

Influence of differences in the distance between the electrodes on the replacement of anions

FRACTIONS	DIALYZATES CHANGED AFTER	ELECTRODE DISTANCE 6 CM.			ELECTRODE DISTANCE 9 CM.			ELECTRODE DISTANCE 12 CM.		
		M.e. per 100 gm. of soil			M.e. per 100 gm. of soil			M.e. per 100 gm. of soil		
		Per fraction	Per hour	Per volt/cm.	Per fraction	Per hour	Per volt/cm.	Per fraction	Per hour	Per volt/cm.
1	2	3	4	5	6	7	8	9	10	11
	hours									
a	12	1.291	0.108	0.071	0.587	0.049	0.048	0.470	0.039	0.051
b	24	0.234	0.020	0.013	0.391	0.033	0.032	0.352	0.029	0.038
c	36	0.120	0.010	0.007	0.199	0.015	0.016	0.274	0.023	0.030
d	48	0.082	0.007	0.004	0.162	0.015	0.013	0.152	0.013	0.017
e	60	0.078	0.007	0.004	0.148	0.012	0.012	0.120	0 010	0.013
f	72	0.078	0.007	0.004	0.144	0.012	0.012	0.112	0.009	0.012
Total.........		1.883	.....	.....	1.631	.....	.....	1.580	.....	.....

circumstance is taken into account can these results give the correct information.

Difference in voltage

The requirement for a great potential difference between the electrodes can also be met by increasing the voltage, by which means the current arises. In laboratories where only 110 volt alternating current is available, direct current can be easily produced and a higher voltage obtained by use of a suitable motor generator set. In the experiments performed by the author a motor generator set furnished with a field rheostat was used for delivering direct current; by use of the rheostat the voltage could be regulated within the range of 80–250 volts. In all experiments the desired voltage was kept constant during the time of experimentation.

Three series of experiments were conducted in order to show the influence of an increased voltage on ion removal. Only two of these, namely, those with soils 14A and 79A, will be reported in detail.

Soil samples of 50 gm. were suspended in a bulb of 450 cc. capacity; the electrode distance was 9 cm. The voltages compared were 125 and 250 with the first mentioned soil, and 110 and 220 with the second soil. These give comparisons between potential differences of 13.9 volt/cm. and 27.8 volt/cm., and between 12.2 volt/cm. and 24.4 volt/cm., respectively.

The analytical data are given in tables 7 and 8. By comparing columns 3 and 7 (table 7) and 3 and 8 (table 8) it is seen that the replacement of cations is much faster with the higher voltage than it is with the lower one. As the voltage is increased from 125 to 250 volts, or from 110 to 220 volts without changing the electrode distance the potential difference is doubled, and it should seem reasonable that the amounts of cations removed from the soils would rise proportionately. The relation, however, is equal to about 1:1½ and not equal to 1:2, presumably because of the gradual increase in the resistance as the cations are removed from the suspension, an increase which will be more pronounced in the cases where the higher voltages are used, because the removal of ions has taken place to a greater extent. For a shorter interval the ratio might have been different.

The numbers given refer to the first fractions, which are entirely comparable. By calculating the amounts of cations liberated per volt/cm. for the a-fractions, the following relationships are found:

Soil 14A:

$$\left.\begin{array}{l}\text{Potential difference 13.9 volt/cm. gave 0.437 M.e. per volt/cm.}\\ \text{Potential difference 27.8 volt/cm. gave 0.344 M.e. per volt/cm.}\end{array}\right\}\text{ or 1:0.79}$$

Soil 79A:

$$\left.\begin{array}{l}\text{Potential difference 12.2 volt/cm. gave 0.301 M.e. per volt/cm.}\\ \text{Potential difference 24.4 volt/cm. gave 0.230 M.e. per volt/cm.}\end{array}\right\}\text{ or 1:0.76}$$

In the rate of anion liberation from the two soils the differences due to increasing voltage are less, which also must be expected since the total amounts of anions removed from the soils are small compared with the amounts of cations.

The higher rate of ionic exchange as a result of increased voltage was also observed in the third series of experiments (with soil 48A), for which the data are not presented.

In connection with the results concerning the liberation of cations it ought to be mentioned that investigation made by Wilson (17) also shows an increasing effect on the rate of replacement by using high voltage in electrodialysis of soils.

It is of interest to note the tendency to a rise in the exchange of cations which takes place after some duration of dialysis of soil 79A, which is shown in table 8. This phenomenon has been observed previously [Odén and Löddesöl (12)], and is supposed to be a consequence of the increase in acidity in the soil suspension during the experiments, followed by a solubility effect on the soil particles. In the experiment where 110 volts is used this rise in cation exchange starts after 108 hours' dialysis, or when about 15 M.e. of bases and about 2 M.e. of acids per 100 gm. of soil are removed from the sample. In the experiment with 220 volts the rise in exchange velocity starts after 96 hours' dialysis or when nearly 20 M.e. of bases and 2.6 M.e. of acids are removed. The pH values of the suspension which were determined at the end of each second interval, however, are in both cases about 5.0 when the rising tendency is observed. As will be understood, the change in pH value of the suspension is not only affected by the amounts of cations liberated from

TABLE 7

Influence of difference in voltage on the rate of removal of ions—soil 14A

FRACTIONS	DIALYZATES CHANGED AFTER	EXPERIMENT WITH 125 VOLTS				EXPERIMENT WITH 250 VOLTS			
		M.e cations per 100 gm. of soil		M e. anions per 100 gm. of soil		M.e. cations per 100 gm. of soil		M.e. anions per 100 gm. of soil	
		Per fraction	Average per hour	Per fraction	Average per hour	Per fraction	Average per hour	Per fraction	Average per hour
1	2	3	4	5	6	7	8	9	10
	hours								
a	12	6.080	0.507	0.720	0.060	9.560	0.797	0.764	0.064
b	24	5.192	0.433	0.210	0.018	6.668	0.556	0.270	0.023
c	36	3.167	0.264	0.183	0.015	2.245	0.187	0.217	0.018
d	48	1.836	0.153	0.139	0.012	1.188	0.099	0.176	0.015
e	60	0.779	0.065	0.115	0.010	0.840	0.070	0.146	0.012
f	72	0.666	0.056	0.141	0.012	0.710	0.059	0.147	0.012
g	84	0.636	0.053	0.102	0.009	0.538	0.045	0.143	0.012
h	96	0.639	0 053	0.094	0.008	0.450	0.038	0.140	0.012
i	108	0.516	0 043	0.086	0.007	0.454	0.038	0.136	0.011
j	120	0.436	0.036	0.074	0.006	0.456	0.038	0.132	0.011
Total..........		19.947	.....	1.864	.....	23.109	.....	2.271	.....

the soil, but also the amounts of anions removed must be taken into consideration. The final pH value for this soil seems to be about 3.90, which was reached in the experiment where 220 volts was applied.

According to Mattson (10a) the appearance of the cations in the cathode chamber is to a large extent affected by the hydrogen-ion concentration in the soil suspension as a result of which weak hydroxides, which at the beginning of the experiment were precipitated on the cathode membrane, are brought into solution. At a certain degree of acidity in the middle compartment the respective cations become mobile and penetrate to the cathode chamber. This statement of Mattson's seems very reasonable in view of the fact that a slight precipitate of iron and aluminum hydroxides has been observed on the cathode membrane in some of the experiments conducted by the author.

TABLE 8

Influence of difference in voltage on the rate of removal of ions—soil 79A

FRACTIONS	DIALYZATES CHANGED AFTER	EXPERIMENT WITH 110 VOLTS					EXPERIMENT WITH 220 VOLTS				
		M.e. cations per 100 gm. of soil		M.e. anions per 100 gm. of soil		pH of the soil suspension	M.e. cations per 100 gm. of soil		M.e. anions per 100 gm. of soil		pH of the soil suspension
		Per fraction	Average per hour	Per fraction	Average per hour		Per fraction	Average per hour	Per fraction	Average per hour	
1	2	3	4	5	6	7	8	9	10	11	12
	hours										
79A*	...	.....	.....	.....	.....	7.40	.....	.....	.....	.....	7.40
a	12	3.680	0.307	0.587	0.049	....	5.600	0.467	1.017	0.085	....
b	24	2.560	0.213	0.391	0.033	6.60	3.444	0.287	0.522	0.044	6.95
c	36	1.786	0.137	0.199	0.015	....	2.371	0.198	0.407	0.034	....
d	48	1.380	0.126	0.162	0.015	6.15	1.772	0.148	0.208	0.017	6.15
e	60	1.360	0.113	0.148	0.012	....	1.755	0.146	0.171	0.014	....
f	72	1.153	0.096	0.144	0.012	5.95	1.476	0.123	0.148	0.012	5.70
g	84	1.123	0.094	0.125	0.010	....	1.364	0.114	0.136	0.011	....
h	96	1.082	0.090	0.121	0.010	5.25	1.598	0.133	0.146	0.012	5.00
i	108	0.971	0.081	0.106	0.009	....	1.845	0.154	0.158	0.013	....
j	120	1.308	0.109	0.114	0.010	4.90	2.052	0.171	0.169	0.014	4.45
k	132	1.584	0.132	0.137	0.011	....	1.802	0.150	0.185	0.015	....
l	144	1.624	0.135	0.154	0.013	4.65	1.451	0.121	0.215	0.018	4.15
m	156	1.700	0.142	0.174	0.015	....	1.090	0.091	0.218	0.018	....
n	168	1.570	0.131	0.153	0.013	4.40	0.956	0.080	0.167	0.014	3.95
o	180	1.282	0.107	0.146	0.012	....	0.819	0.068	0.149	0.012	....
p	192	0.972	0.081	0.115	0.010	4.15	0.632	0.053	0.117	0.010	3.92
Total.......		25.135	.....	2.976	.....	....	30.027	.....	4.113	.....	....

* Undialyzed soil.

Different membranes

The general advice for use of membranes in electrodialysis experiment is that negative membranes shall be used at the cathode and positive membranes at the anode. The main reason for such an arrangement is that the penetration of ions is facilitated when the membranes have an electrical charge opposite to that of the ions which are transported to the respective electrodes. In other words, negatively charged membranes are more permeable for cations than they are for anions, and if we deal with positively charged membranes the permeability for anions is greater than for cations.

The influence of different membranes on the rate of migration of different ions by electrodialysis has been studied by Bradfield (6, 7) using solutions of different sodium salts as a medium. He found, by placing a negatively charged membrane (parchment) at the cathode and a positively charged membrane (hemoglobin-collodion) at the anode, that the movement of cations and anions from the middle compartment to the respective electrode chambers took place in about equivalent amounts, and that the reaction of the solution in the central compartment changed to only a small extent. By using negatively charged membranes on both sides, however, he found that the movement of cations was much greater than that of the anions, followed by an increase in the hydrogen-ion concentration in the middle chamber. By adding a suspension of electrodialyzed colloidal clay to different solutions of dialyzable salts, Bradfield found that the behavior of the anode membrane changed as the rate of the removal of anions was retarded, because a layer of negatively charged clay colloids was formed on the inside of the membrane.

By continued electrodialysis of a soil suspension a gradual change in reaction in the acid direction takes place as a result of the replacement of the adsorbed base cations by hydrogen ions. In the previous tables it is seen that the amounts of anions liberated from the soils are much less than the amounts of cations. This is only what we could expect since most of the cations liberated have been associated with the acidoid soil complexes which remain in the middle compartment because the membranes are impermeable to colloids.

The extent to which the kind of membranes affects the rate of liberation of ions from a soil suspension has been but little investigated. Odén and Wijkström (13) recommend parchment, which is negatively charged, as cathode membrane; and cellophane, which the authors state is positively charged, as anode membrane. Then the electroendosmosis which takes place in the system will support the movement of ions and the process will be facilitated.

In a series of experiments conducted by the author parchment has been used both as cathode membrane and anode membrane in comparison with parchment used as cathode membrane and cellophane as anode membrane. The influence of changing the anode membrane on the speed of anion and cation liberation in relation to the change in the hydrogen-ion concentration in the soil suspension was then investigated.

The results of experiments with soil 7A are given in table 9. Soil samples of 50 gm. were used and dialyzed in Bradfield's three-compartment cell for 150 hours with a voltage of 110. By comparison of the first fractions in the table it will be seen that the liberation of anions takes place to a greater extent when the anode membrane consists of cellophane instead of parchment (columns 5 and 10), but at the same time the amount of cations is less (columns 3 and 8). The results indicate that cellophane is more permeable to anions than parchment and this behavior might be explained as a result of the different charge of the two kinds of membrane. The explanation for the greater quantity of cations liberated in the case where parchment is used as anode membrane must be that an accumulation of anions in the middle chamber, followed by a formation of acids, has caused an increased solubility effect on the soil particles. The pH determinations made in the soil suspension from interval to interval (columns 7 and 12) seem also to indicate that anions must have been accumulated in the middle chamber to a certain extent during the first intervals when parchment was used as anode membrane, because the lower pH values found can hardly be due only to the slight amounts of cations which are removed. The end pH values as well as the total amounts of ions liberated were in both experiments about the same.

TABLE 9

Influence of different membranes on the rate of removal of ions—soil 7A

FRACTIONS	DIALYZATES CHANGED AFTER	PARCHMENT BOTH AS CATHODE MEMBRANE AND ANODE MEMBRANE					PARCHMENT AS CATHODE MEMBRANE AND CELLOPHANE AS ANODE MEMBRANE				
		M.e. cations per 100 gm. of soil		M.e. anions per 100 gm. of soil		pH of the soil suspension	M.e. cations per 100 gm. of soil		M.e. anions per 100 gm. of soil		pH of the soil suspension
		Per fraction	Average per hour	Per fraction	Average per hour		Per fraction	Average per hour	Per fraction	Average per hour	
1	2	3	4	5	6	7	8	9	10	11	12
	hours										
7A*	...	.....	.....	.....	.....	5.35	.....	.....	.....	.....	5.35
a	12	1.454	0.121	0.365	0.030	5.05	1.414	0.118	0.446	0.037	5.20
b	24	0.341	0.028	0.132	0.011	4.70	0.322	0.027	0.166	0.014	5.00
c	36	0.269	0.022	0.106	0.009	4.32	0.251	0.021	0.099	0.008	4.90
d	48	0.144	0.012	0.104	0.009	4.30	0.177	0.015	0.088	0.007	4.72
e	60	0.144	0.012	0.098	0.008	4.28	0.097	0.008	0.073	0.006	4.58
f	72	0.051	0.004	0.093	0.008	4.22	0.089	0.007	0.064	0.005	4.36
g	84	0.054	0.005	0.089	0.007	4.20	0.071	0.006	0.059	0.005	4.32
h	100	0.044	0.003	0.098	0.006	4.20	0.053	0.003	0.072	0.005	4.23
i	125	0.068	0.003	0.113	0.005	4.20	0.093	0.004	0.108	0.004	4.25
j	150	0.065	0.003	0.104	0.004	4.18	0.074	0.003	0.100	0.004	4.23
Total.......		2.634	.....	1.302	.....	....	2.641	.....	1.275	.....	....

* Undialyzed soil.

Experiments with soil 50A gave similar results as those stated for soil 7A. The use of cellophane as anode membrane had facilitated the dialysis of anions during the first interval but at the same time the amounts of cations were less than when parchment was used.

The total amounts of exchangeable cations in the soils 7A and 50A are very low. In order to see the effect of cellophane compared with parchment as anode membrane also on alkaline soil rich in exchangeable cations experiments with soil 79A were performed. The conditions under which the experiments were carried out were the following: Samples of 100 gm. of soil were suspended in a bulb of 550 cc. volume, the electrode distance was 12 cm. and the voltage 110.

According to table 2, soil 79A is rather high in organic matter, which adsorbs and holds cations very strongly (12). It was assumed that the exchange velocity of ions from such a soil would be slow, and the conditions under which the experiments were set up, particularly the large samples and the long electrode distance in relation to the voltage, would assure a very slow rate of replacement. That was also especially desired in this case where the relation between the ion liberation and the change in reaction of the soil suspension should be studied.

TABLE 10

Influence of different membranes on the rate of removal of ions—soil 79A

FRACTIONS	DIALYZATES CHANGED AFTER	PARCHMENT BOTH AS CATHODE MEMBRANE AND ANODE MEMBRANE					PARCHMENT AS CATHODE MEMBRANE AND CELLOPHANE AS ANODE MEMBRANE				
		M.e. cations per 100 gm. of soil		M.e. anions per 100 gm. of soil		pH of the soil suspension	M.e. cations per 100 gm. of soil		M.e. anions per 100 gm. of soil		pH of the soil suspension
		Per fraction	Average per hour	Per fraction	Average per hour		Per fraction	Average per hour	Per fraction	Average per hour	
1	2	3	4	5	6	7	8	9	10	11	12
	hours										
79A*	...	.....	.....	.....	.....	7.40	.....	.....	.....	.....	7.40
a	12	1.620	0.135	0.332	0.028	7.22	1.700	0.142	0.391	0.033	7.30
b	24	1.530	0.128	0.160	0.013	7.12	1.510	0.126	0.220	0.018	7.18
c	36	1.435	0.120	0.122	0.010	7.08	1.290	0.108	0.122	0.010	7.05
d	48	0.975	0.081	0.103	0.009	7.02	0.742	0.062	0.103	0.009	7.00
e	60	0.713	0.059	0.073	0.006	6.97	0.728	0.061	0.085	0.007	6.92
f	72	0.567	0.047	0.078	0.007	6.80	0.698	0.058	0.073	0.006	6.80
g	84	0.560	0.047	0.084	0.007	6.60	0.599	0.050	0.070	0.006	6.65
h	96	0.547	0.046	0.081	0.007	6.52	0.565	0.047	0.062	0.005	6.53
i	108	0.529	0.044	0.073	0.006	6.45	0.502	0.042	0.058	0.005	6.47
j	120	0.515	0.043	0.074	0.006	6.37	0.487	0.041	0.059	0.005	6.35
k	132	0.504	0.042	0.076	0.006	6.30	0.468	0.039	0.055	0.005	6.27
l	144	0.484	0.040	0.072	0.006	6.25	0.452	0.038	0.051	0.004	6.25
m	150	0.295	0.049	0.049	0.008	6.20	0.290	0.048	0.044	0.007	6.22
Total.......		10.274	.....	1.377	.....	....	10.031	.....	1.393	.....	....

* Undialyzed soil.

The results are given in table 10. For this soil, also, the amounts of anions removed during the first intervals are greater in the case where cellophane is used as anode membrane (columns 5 and 10) than where parchment is used. Regarding the liberation of cations it is of interest to note that in the case of the most alkaline reaction the larger amount is found in the experiment with cellophane as anode membrane, but as soon as the reaction changes to about neutral or becomes acid a tendency in the opposite direction is observed (columns 3 and 8). The changes in the pH values are seen to be closely related to the amounts of ions removed from the suspensions (columns 7 and 12). The experiments have not been run long enough to reach the point of increased acidity where an increased replacement of cations take place (table 8). The somewhat greater quantities of ions found in the last fractions (the m-fractions) in relation to previous fractions are evidently a result of changing the dialyzates after a shorter period of time than usual (column 2).

TABLE 11

Influence of different membranes on the rate of removal of ions—soil 48A

FRACTIONS	DIALYZATES CHANGED AFTER	CELLOPHANE BOTH AS CATHODE MEMBRANE AND ANODE MEMBRANE					CELLOPHANE AS CATHODE MEMBRANE AND PARCHMENT AS ANODE MEMBRANE				
		M.e. cations per 100 gm. of soil		M.e. anions per 100 gm. of soil		pH of the soil suspension	M.e. cations per 100 gm. of soil		M.e. anions per 100 gm. of soil		pH of the soil suspension
		Per fraction	Average per hour	Per fraction	Average per hour		Per fraction	Average per hour	Per fraction	Average per hour	
1	2	3	4	5	6	7	8	9	10	11	12
	hours										
48A*	...	.....	.....	.....	.....	6.25	.....	.....	.....	.....	6.25
a	12	1.880	0.157	0.368	0.031	6.05	1.600	0.133	0.400	0.033	6.00
b	24	1.339	0.112	0.260	0.022	5.85	1.394	0.116	0.213	0.018	5.80
c	36	0.820	0.068	0.131	0.011	5.30	0.888	0.074	0.201	0.017	5.28
d	48	0.724	0.060	0.091	0.008	5.00	0.760	0.063	0.228	0.019	4.90
e	60	0.886	0.074	0.059	0.005	4.65	0.681	0.057	0.204	0.017	4.60
f	72	0.946	0.079	0.077	0.006	4.30	0.605	0.050	0.216	0.018	4.25
g	84	0.796	0.066	0.106	0.009	4.25	0.569	0.047	0.201	0.017	4.22
h	96	0.675	0.056	0.140	0.012	4.20	0.580	0.048	0.201	0.017	4.20
i	108	0.545	0.045	0.145	0.012	4.18	0.675	0.056	0.198	0.017	4.20
j	120	0.508	0.042	0.129	0.011	4.20	0.733	0.061	0.183	0.015	4.15
Total.......		9.119	.....	1.506	.....	....	8.485	.....	2.245	.....	....

* Undialyzed soil.

Two series of experiments were also performed where cellophane was used both as cathode and anode membranes compared with cellophane as cathode membrane and parchment as anode membrane. Samples of 50 gm. of soil 48A and 14A were dialyzed in the author's apparatus with a voltage of 250 for 120 hours. The experiments with the two soils gave similar results, therefore detailed data will be reported only for soil 48A.

Table 11 gives the experimental data. There seems to be but little difference in the amounts of ions liberated whichever of these two arrangements is used, but it ought to be mentioned that the removal of ions from the soil in both cases is very slow. This can be seen by the data in columns 4 and 6 and in columns 9 and 11, which give the average amounts of ions liberated per hour in the various intervals. The numbers given in the columns mentioned show that the quantities of ions which are liberated from one interval to the other decrease very slowly. The reason for this must undoubtedly be the incorrect placing of the membranes.

Columns 7 and 12, which give the pH values at the end of each interval, show a very good accordance in the change of the reaction of the soil suspension in the two experiments. The final pH value for this soil seems to be about 4.20. These values were obtained with small samples taken out of the middle chamber containing the stirred suspension. It is of interest, however, that determination of the pH value of a suspension made of clay colloids which were transported toward the anode and precipitated on the anode membrane shows the pH-value 3.45 [Bradfield (5)].

In order to find the total amounts of ions transported from the suspensions by use of different membranes the corresponding amounts of cations and anions were added and the sums are shown in the following collocation. As will be understood, only the a-fractions are entirely comparable:

Soil 7A: Cathode membrane parchment, anode membrane parchment, sum = 1.519 M.e.
Soil 7A: Cathode membrane parchment, anode membrane cellophane, sum = 1.859 M.e.
Soil 79A: Cathode membrane parchment, anode membrane parchment, sum = 1.952 M.e.
Soil 79A: Cathode membrane parchment, anode membrane cellophane, sum = 2.091 M.e.
Soil 48A: Cathode membrane cellophane, anode membrane parchment, sum = 2.000 M.e.
Soil 48A: Cathode membrane cellophane, anode membrane cellophane, sum = 2.216 M.e.

The greater quantities of ions transported when cellophane was used as anode membrane indicate that this material should be preferred in comparison with parchment. The ammeter reading during the experiments also showed that the current rose to higher values and the maximum amperage was obtained after shorter periods of time, which indicates that the resistance due to the anode membrane itself was less in the cases where cellophane was used.

TOTAL AMOUNTS OF EXCHANGEABLE CATIONS IN THE SOILS USED IN THE INVESTIGATION

In table 12 are summarized results of experiments for the six soils which were used in the investigations. Only the experiments in which the replacement of the exchangeable cations was supposed to have been completed, or at least nearly completed, are reported in the table.

It is of interest to note that the influence of the different treatments is most pronounced in the beginning of the experiments, but little by little the amounts of cations liberated from the same soil tend to be of the same magnitude. The quantities of cations given off, however, increased as long as the experiments continued, and it is of interest to know at what time the process of electrodialysis of a given sample is finished. How far the final values in the table really represent the so-called exchangeable or adsorbed cations in the soils is therefore an open question which requires further examination.

It might here be desirable to call attention to the fact that the terms "exchangeable" or "replaceable" cations very often are used as designations for all the cations liberated from soils regardless of whether or not the sources are adsorbed cations or cations from easily soluble salts. In many cases, especially for soils from humid regions, the last mentioned part is small compared to the former, but if any, this amount presumably will be liberated in the beginning of the experiments. Also by the neutral salt extraction methods at least the water-soluble salts are involved in the amounts taken out by the leaching procedure, provided the soil has not been washed with water for a sufficient time beforehand.

TABLE 12

Total amounts of electrodialyzable cations in the soils used in the investigation

SOIL NUMBER	DIFFERENCE IN THE EXPERIMENTAL CONDITIONS	TIME DIALYZED	M.E. CATIONS PER 100 GM. OF SOIL AFTER:					
			12 hours	24 hours	36 hours	60 hours	100 hours	150 hours
		hours						
	Volusia silt loam							
7A	Anode membrane parchment	150	1.454	1.795	2.064	2.352	2.501	2.634
	Anode membrane cellophane	150	1.414	1.736	1.987	2.281	2.474	2.641
49A	25 gm. sample	100	2.474	2.918	3.124	3.278	3.465	.....
	50 gm. sample	125	1.689	2.564	2.911	3.270	3.545	3.693*
50A	25 gm. sample	100	2.926	3.633	3.957	4.339	4.650	.....
	50 gm. sample	150	2.239	3.219	3.702	4.186	4.467	4.701
			36 hours	60 hours	96 hours	120 hours	144 hours	192 hours
	Ontario loam							
14A	Voltage = 125	120	14.439	16.054	18.995	19.947	......	.....
	Voltage = 250	120	18.473	20.501	22.199	23.109	.....	.....
48A	Anode membrane cellophane	120	4.039	5.649	8.066	9.119	.....	.....
	Anode membrane parchment	120	3.882	5.323	7.077	8.485	.....	.....
79A	Voltage = 110	192	8.026	10.766	14.124	16.403	19.611	25.135
	Voltage = 220	192	11.415	14.942	21.380	25.277	28.530	30.027

* 125 hours.

Besides cations from eventually easily soluble salts the electrodialysis as well as the leaching methods are supposed to cause a solubility or weathering effect on the soil particles themselves, so that the results obtained also include more or less cations from more difficultly soluble salts and the soil complexes.

Most writers who deal with electrodialysis of soils claim that their experiments have been continued till a constant amperage is obtained, or to at least one hour after the dialyzate drained from the cathode chamber does not give a color with phenolphthalein. The solubility effect during the time of experimentation is then not taken into consideration. Odén and Wijkström (13), however, point out the necessity of distinguishing between the exchangable cations and cations liberated by weathering, and have found that the last quantity follows closely the parabolic equation:

$$y = B + \beta\sqrt{t}$$

from the time the replacement of the adsorbed base cations is completed· The y in the formula is the total amount of cations in M.e. liberated from the soil, B is the supply of cations which are exchangeable and those which are present in the soil solution developed from soluble salts, t is the time in hours, and β represents the weathering velocity of the soil. The reported equation is tried and found valid for calcium, magnesium, potassium, and sodium; by addition of the single equations the total amount of base cations (Y) from the respective soils can be found.[3]

The amount of cations liberated as a result of solubility processes might increase during the time of dialysis as a result of the increasing acidity in the soil suspension, although the differences in quantities of ions liberated under somewhat different reactions are not found to be very great (cf. the membrane experiments). On the other hand, it should seem reasonable that the amounts would decrease with time since the previously most weathered particles and part of the particles disappear first and the unweathered minerals will be left. The easily soluble salts will go into solution and the cations removed from the soil suspension to the greatest extent in the beginning of the experiments. Taking these circumstances into consideration the electrodialyzable cations appear to consist of the adsorbed cations plus a smaller amount of cations successively liberated from soluble salts and by weathering effect on the soil complexes, and it might not be far from the truth to assume that the amounts which are liberated from the last mentioned sources are approximately the same from time to time during the whole experiment. If this quantity could be found and subtracted from the total amount the remainder should be the exchangeable cations.

When a point of minimum cation liberation is reached the amounts of cations which are still liberated from the soil suspension remain approximately constant from interval to interval (cf. previous tables). All these cations presumably are removed as a result of the dissolving processes which act in the suspension. If these processes have been acting as long as the experiment has run and to about the same degree of effectiveness the correction should be the average amount of cations liberated per hour, after a constant value per time unit is obtained, multiplied by the number of hours of the experiment. This product subtracted from the total sum should give the exchangeable cations. This correction is related to that of Hissink (8) in his extraction method where the amount of base cations found in the second liter of filtrate is subtracted from the amount found in the first liter. The basis for this manner of proceeding in the Hissink's method is the assumption that the solution process goes on during the whole time of leaching to approximately the same extent. Compared with Odén and Wijkströms equation, and using the same symbols, the suggested correction will give a straight line instead of a parabola from the moment all exchangeable cations are removed from the soil, and the factor B will not compose the cations from the soluble salts (which here are included in the factor β), but only the adsorbed or exchangeable cations.

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[3] Cf. also a previous paper by Oden (11) where this problem is discussed.

By examination of the tables giving the detailed results of the experiments it is found that only for soils 7A, 49A, 50A, and the high voltage experiment with soil 14A, are the points reached where the liberation of cations remains approximately constant during more intervals, and where at the same time approximately constant amperage is obtained. A constant amperage should, to a certain extent, indicate that the amounts of cations transported from time to time remain constant. In the experiments with soils 48A and 79A it is evident that the points of constant cation liberation are not reached, facts which also were observed during the experimentation, since constant ammeter readings were not obtained before the experiments were finished. The correction can therefore be tried only on four of the six soils studied. The results of such a trial are given in table 13, and the numbers given in column 7 should be the amounts of exchangeable cations in the soils.

TABLE 13

Total amounts of exchangeable cations in some of the soils studied

SOIL NUMBER	LENGTH OF EXPERIMENTS	FINAL CATION LIBERATION PER HOUR	CORRECTION	TOTAL AMOUNTS OF CATIONS IN THE SOILS	EXCHANGEABLE CATIONS	MEAN	REFERENCE TABLE NUMBER
1	2	3	4	5	6	7	8
	hours	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*	
7A	150	0.003	0.450	2.634	2.184	2.188	9
	150	0.003	0.450	2.641	2.191		9
49A	100	0.005	0.500	3.465	2.965	2.954	3
	125	0.006	0.750	3.693	2.943		3
50A	100	0.006	0.600	4.650	4.050	4.001	4
	150	0.005	0.750	4.701	3.951		4
14A	120	0.038	4.560	23.109	18.549	18.549	7

In figure 1 the relationship between cation liberation and amperage is shown for soil 14A. The time is plotted on the abscissae and the total amounts of cations liberated from time to time on the ordinates. The amperage during the experiment is also plotted on the ordinates. The amounts of cations are given in milliequivalents and the amperage in milliamperes.

The connection between approximately constant cation liberation and amperage is clearly indicated from the graph. The correction suggested can also be illustrated graphically. If a tangent, which coincides with the straight part of the curve, is extended to the y-axis this axis will be cut at a certain point. The distance from the origin to this point represents the exchangeable cations.

It must be pointed out, however, that the different cations are set free with different speeds during the time of electrodialysis (12). Therefore a correction which might afford some aid in the estimation of the total amounts of exchangeable cations is perhaps less useful for the single cations.

If the problem is an investigation and comparison of the amounts of readily available cations from a number of soils a distinction between exchangeable cations and cations liberated by solution and weathering is of more theoretical interest than of practical value, because it is the total quantity of readily available base cations which indicates whether or not the soils are rich in plant nutrients. On the contrary, from the standpoint of comparison of the quantities of cations from different soils it is of great importance that the determinations are performed under similar and known conditions, such that the results obtained really are comparable.

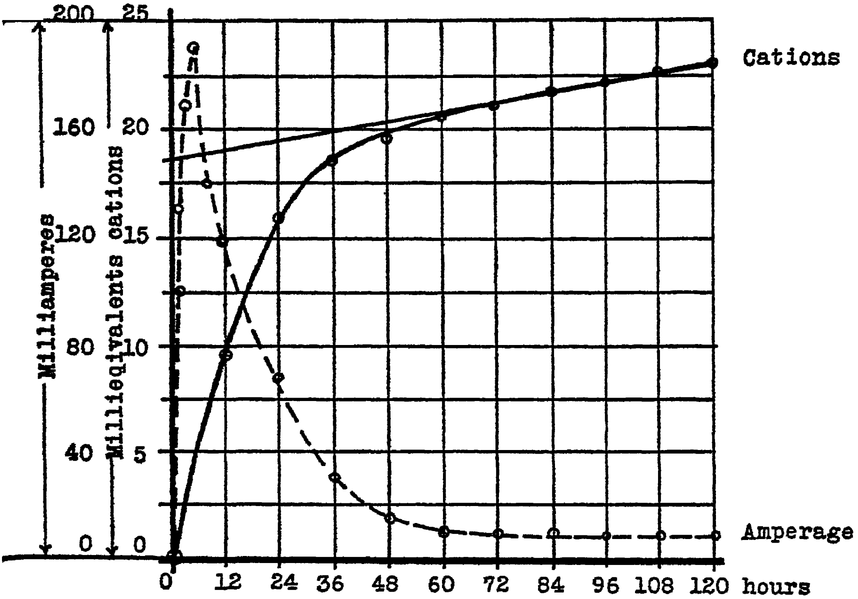

FIG. 1. RELATION BETWEEN CATION LIBERATION AND AMPERAGE

In order to verify the results from the electrodialysis experiments the amounts of exchangeable cations in the soils were also, as previously mentioned, determined by extraction with ammonium acetate. These results are given in table 14. The table gives also the amounts of cations from water-soluble salts in the soils.

As seen from the table the amounts of cations obtained by water extraction are small for all soils. The results of the ammonium acetate extraction show that leaching of the samples until 200 cc. of filtrate are obtained, such as proposed by Baver (2), has not been sufficient for these soils, especially for the soils which are rich in exchangeable cations (cf. soils 14A and 79A). This appears from columns 4, 5, and 6, which show that the second fractions of filtrate contain more cations than do the third and fourth fractions. For soils rich in replaceable cations the leaching procedure therefore apparently should be continued at least until 500 cc. of filtrate are obtained. It may also be seen from the data presented that ammonium acetate solution caused a slight solubility effect in the soils. In order to obtain the amounts of exchangeable cations as accurately as possible the cation contents of the a- and b-fractions are added and then corrections for solubility are made by subtracting the amounts of cations in the c- and d- fractions (column 8), and finally the cations from water-soluble salts are subtracted (column 9). The numbers in column 9 should consequently be the amounts of exchangeable cations.

TABLE 14

Results from the extraction experiments

SOIL NUMBER	M.E. CATIONS PER 100 GRM. OF SOIL							
	Water extraction	Ammonium acetate extraction					Reduced total [(a + b) − (c + d)]	Reduced total minus water soluble
		a	b	c	d	Total		
1	2	3	4	5	6	7	8	9
7A	0.249	2.538	0.137	0.117	0.137	2.929	2.421	2.172
49A	0.117	3.318	0.193	0.097	0.077	3.681	3.337	3.220
50A	0.225	4.490	0.176	0.117	0.137	4.920	4.412	4.157
14A	0.176	16.836	1.562	0.390	0.195	18.983	17.813	17.637
48A	0.215	7.320	0.117	0.078	0.098	7.613	7.261	7.046
79A	0.156	24.298	3.074	1.171	0.683	29.226	25.518	25.362

Comparisons between the amounts of "exchangeable cations" found by electrodialysis (I) and by ammonium acetate extraction (II) give the following results:

	I (Table 13)	II (Table 14)
Soil 7A	2.188 M.e.	2.172 M.e.
Soil 49A	2.954 M.e.	3.220 M.e.
Soil 50A	4.001 M.e.	4.157 M.e.
Soil 14A	18.549 M.e.	17.637 M.e.

As may be seen, the agreement between the two methods is good for these soils when in both cases corrections are made for the solubility effect. The parabolic equation referred to previously, has also been applied to the soils described, but in these cases it gives too small reductions of the total amounts of cations found if the ammonium acetate method is used as standard. The data presented by Odén and Wijkström (13) show, however, that the equation given by these authors seems to be a very good mathematical expression of the process of weathering caused by electrodialysis.

According to table 14, the amounts of exchangeable cations for soil 48A are 7.046 M.e., and for soil 79A, 25.362 M.e. From table 12 it may be seen that the total amounts of cations found for these soils in the electrodialysis experiments are greater than the sums mentioned. The surplus must be results of solubility effect during the experimentations, but, as previously stated, these experiments were not continued long enough to reach constant cation liberation such that corrections can be made.

It is of interest to call attention to the great difference in the amounts of exchangeable cations which are found for the two types of soils used in this investigation. According to table 1, which shows the total amounts of the most important base cations in the soils, the Volusia silt loam soils should contain but little less of cations than the Ontario loam soils. In the content of exchangeable cations, however, the difference is great in favor of the Ontario loam soils. The Ontario loam is known as a very productive type of soil in comparison to the Volusia silt loam (9, p. 110) and one of the reasons why might be the greater content of easily available base cations.

Checking the results from the electrodialysis experiments as well as the ammonium acetate extractions performed gave very good agreement for experiments made under similar conditions. The previously mentioned verification of the determinations of the total amounts of cations from the extraction experiments as chlorides by electrodialysis of aliquots of the filtrate and titration of the bases in the cathode water gave practically the same results as the $AgNO_3$ titration.

SUMMARY

This paper presents results from a series of electrodialysis experiments performed in order to show the influence of various experimental conditions on the speed of cation liberation from certain soils. The conditions investigated are differences in the concentration of the soil suspension, differences in the distance between the electrodes, differences in voltage, and differences in membranes. The results are given in connection with the description of the various experiments.

Determinations of the total amounts of exchangeable cations in the investigated soils by extraction with ammonium acetate were also made. The results are compared with the results from the electrodialysis experiments and an attempt is made to distinguish between the total quantities of cations liberated from the soils and the amounts of exchangeable cations.

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PLATE 1

APPARATUS USED IN EXPERIMENTS

FIG. 1. The author's electrodialysis apparatus
FIG. 2. Mechanical dispersion and stirring equipments

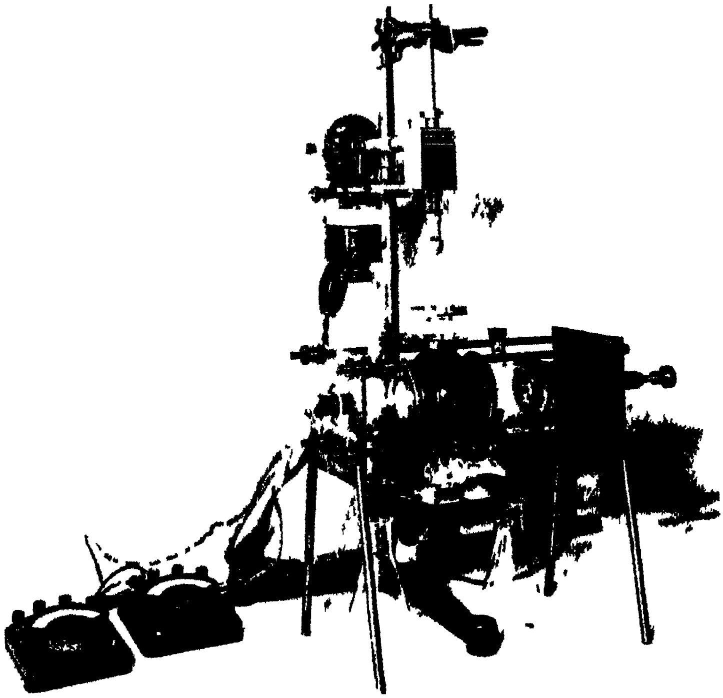
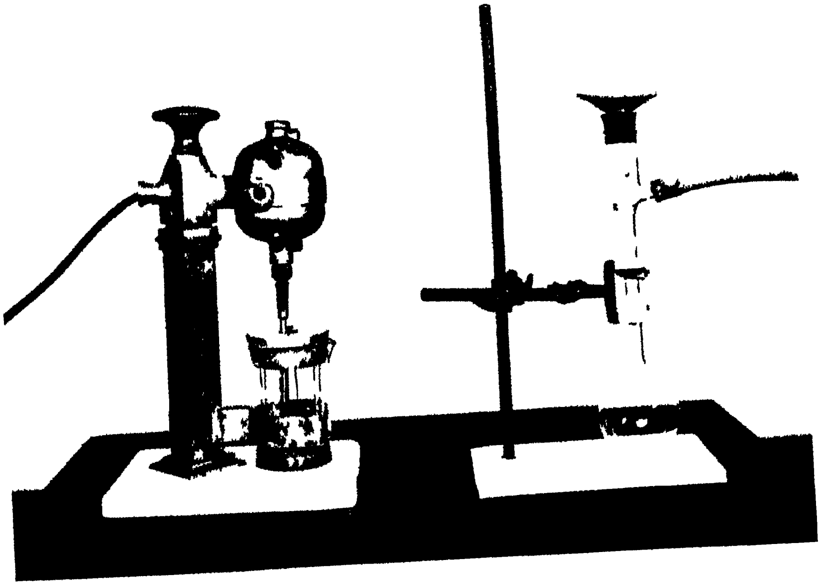


Fig. 1



THE DETERMINATION OF NITRATES IN SOILS CONTAINING SOLUBLE ORGANIC MATTER

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The determination of nitrates by the phenoldisulfonic acid method in humus materials, or in soils which contain soluble organic matter, is often made difficult or impossible by discoloration caused by the organic matter. This is especially true when the nitrate content of the material being studied is low. If the nitrate content of the material is fairly high, such as occurs in some muck soils after incubation, the discoloration may be disregarded if it is not too intense.

The writer, while studying forest soils in which the nitrate content is usually low, found that nearly all samples of litter and pronounced duffs yield water solutions which are too highly colored to determine even approximately the quantity of nitrate nitrogen present, using the ordinary methods of procedure. Such clarifying agents as aluminum and copper hydroxides, bone black, and G-elf carbon black mainly remove material in suspension. Soluble discoloring materials are not greatly affected by them.

A method suggested by Syme (2), which involves the oxidation of the soluble organic material by means of potassium permanganate, has proved ineffectual. This is mainly because of the difficulty in adding exactly the right amount of oxidizing reagent, the least excess of which discolors the solution still further. Also, after the permanganate has acted upon the organic matter a residue remains, which is difficult to filter off.

A simple and convenient method which has proved to be very satisfactory, giving clear and uncolored solutions in practically every instance, involves the use of concentrated hydrogen peroxide (superoxol) and ammonium hydroxide. The procedure is as follows:

A soil-water mixture is prepared in the usual manner, but without the addition of a precipitating agent. After the mixture is filtered a little powdered $Ca(OH)_2$ (0.5 gm. to 100 cc. solution) is added to the filtrate. If the hydroxide is added to the soil-water mixture before filtering, the discoloration is accentuated in many cases. The filtrate should be stirred after the addition of the hydroxide and allowed to stand for several minutes and then filtered. This procedure removes much, if not most, of the coloring matter from the solution. Often, however, some discoloration will remain.

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[1] Department of forest soils research.

An aliquot of the filtrate is placed in a porcelain dish and evaporated to dryness. If any soluble organic matter is present the residue or incrustation in the bottom of the dish will be brownish in color, the intensity of which depends upon the nature and amount of the organic matter present. The evaporating dish is allowed to cool and approximately 0.5 cc. of 1:1 ammonium hydroxide is added. The dish is then rotated carefully in order to wet thoroughly all of the residue. Five to fifteen drops of superoxol are then added, according to the intensity of the brown color, and the dish is again rotated until the superoxol is in thorough contact with all of the residue. The reaction may be accelerated by placing the dish on a steam bath and carefully evaporating the solution to dryness. If any discoloration remains the residue should again be wetted with the ammonia and superoxol and evaporated. If a discoloration should still persist, it is most probably due to traces of iron or manganese and not due to organic matter. Aluminum and silica may also be present. In either case further attention need not be given to the color because these substances will not interfere with the final reading operation when comparison with the standard color is made. The residue is finally wetted with ammonia and evaporated to dryness in order to expel any excess of the superoxol. From this point the regular procedure is followed.

TABLE 1

Nitrates recovered from soils containing known amounts of nitrates

KNOWN AMOUNT OF NITRATES PRESENT	TOTAL NITRATES RECOVERED							
	Treated				Untreated			
	1	2	3	4	1	2	3	4
p.p.m.								
1	1	1	1	1−	1+	1	1	1
3	3	3	3−	3+	3	3	3+	3
5	5−	5	5	5	5	5+	5−	5
10	10	10+	10+	10+	10	10	10−	10
20	19	20	20	19	20	20	20	19
30	30	30	31	31	30	29	30	30
50	49	51	49	50	50	48	50	50
100	101	99	98	101	100	101	101	101
300	297	300	298	302	302	299	298	300
500	496	495	503	501	498	501	500	498
1,000	1,000	1,005	994	1,003	998	997	1,001	1,002

Most of the discoloration due to soluble organic matter can also be removed by superoxol after the yellow nitrate color has been developed in the ordinary manner. This procedure is not as effective as the one outlined in the foregoing and requires the use of much more superoxol.

In order to determine whether any inaccuracy is caused by the use of superoxol, i.e., whether nitrates are formed or destroyed, tests were made using known amounts of nitrates. Satisfactory checks were obtained in all instances, whether the nitrate content was high or low, as shown in table 1.

In the same manner it was found that nitrites present in the soil solution would not be oxidized to nitrates to any determinable extent. Nitrites are extremely unstable in the presence of strong ammonia and are almost immediately lost in the form of a gas or a mixture of gases. Nitrites are also unstable in the presence of strong acid and would be lost in a similar manner when phenoldisulfonic acid is added to the residue in the regular procedure. Gaarder and Hagem (1) in Norway, write that contrary to most investigators, they find that nitrites in soil solutions are oxidized to nitrates when treated with the phenoldisulfonic acid.

It was found, however, that when large amounts of nitrites were used—several hundred parts per million—a slight oxidation of nitrites to nitrates occurred because, a slight increase over the known amount of nitrates present was obtained. Since soils rarely contain more than traces of nitrites, it is believed that the method here outlined is very accurate and practicable for the determination of nitrates in soils which contain soluble organic matter.

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SOIL PROFILE STUDIES: IV. MORPHOLOGICAL AND CHEMICAL EVIDENCE OF PODZOLIZATION

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THE A_0 LAYER

The general morphological and chemical features of the A_0 layer in a typical podzol profile on a loamy parent material are as follows:

A_0 is the humus-decay accumulative layer which consists of leaf mould and forest litter, about 2 to 3 or more cm. thick, depending on conditions referred to in a previous publication (2). It corresponds to what the foresters refer to as the F and H layers. It is intermingled with some mineral soil material. This layer is accumulative in the sense that the organic matter of plant and animal origin upon decay leaves behind humus substances which are decomposition products and newly synthesized complexes of biological and chemical origin.

The carbon, oxygen, nitrogen, and hydrogen which make up the bulk of the organic matter originate in the atmosphere and hydrosphere. And it is through the organic matter that the atmosphere and hydrosphere react with the aid of the biosphere agency on the lithosphere in the process of soil formation.

That portion of the completely decomposed—in other words, mineralized—organic matter from the humus accumulative layer leaves behind mineral substances. These minerals originally come from the lower horizons through the agency of growth and in that way A_0 is genetically related to the entire body of the soil profile.

Mineralization process

A portion of the minerals combines with the humus substances, forming the organic complexes such as Ca, Mg, Mn, Fe, and Al humates. Because of the carbonic acid formed in the process of decomposition, the nitric acid—in the nitrification process, sulfuric acid—in the oxidation of sulfur compounds, and the organic acid as split products in the process of organic matter decomposition, some of the bases in the humate complexes are replaced by hydrogen and the material of the A_0 is therefore almost always acid. Some of the hydrogen saturated complexes become mobile because of their high dispersion and they move downward. With them move the other portion of the minerals and the free acids. They react with the mineral constituents of the underlying horizon, decompose the silicates, and release the bases which join the substances from the A_0 in a further downward movement.

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[1] Journal series paper of the New Jersey Agricultural Experiment Station, department of soil chemistry and bacteriology.

Thus A_0 is the starting point of many reactions. It is the potential source and storehouse of the substances which react with the underlying horizons and impart to the profile the characteristic features of podzols.

Base-exchange capacity

One of the distinctive chemical properties of A_0 is its high base-exchange capacity, especially when compared with the same property in the other horizons. In no zonal soil type is the difference in base exchange capacity between the A_0 and the underlying horizons so great as in the podzol zone.

TABLE 1

*Some chemical properties of the A_0 layer**

SOIL SERIES	DEPTH OF A_0	REACTION		BASE EXCHANGE CAPACITY	UNSATURATION		BASE EXCHANGE CAPACITY OF HORIZON C
		H_2O extract	Neutral salt extract				
	cm.	*pH*	*pH*	*m.e.*†	*m.e. of H*	*per cent*	*m.e.*
Sassafras loam	3	5.0	3.8	22.3	19.7	88.3	5.2
Penn silt loam	3	5.2	4.3	26.4	18.64	70.6	11.2
Chester gravelly loam	3	6.2	5.0	18.5	5.83	31.5	5.3
Washington loam	5	7.2	6.8	37.2	1.98	5.3	5.03

* The soils on which the analyses are presented are not true podzols. They are members of the podzol zone, the southern belt variety, which was discussed in an earlier publication (2).

† M.e. is the milligram equivalents per 100 gm. of soil. The Ba-acetate method was used in determining the base-exchange capacity and the unsaturation.

Another distinctive chemical property of A_0 in the podzols is its high unsaturation.

In table 1 a few properties of A_0 in a number of soils in New Jersey are presented.

The data in table 1 show that the base-exchange capacity of A_0 is fairly high. For comparison the exchange capacity of the parent material (horizon C) is given. The latter is taken as a measure of the base-exchange capacity of the mineral portion of the soil. As a matter of fact, it is difficult to determine the constant for the base-exchange value of the mineral fraction which should be applicable to all the profile horizons, even in any one particular soil. It is variable because of the dynamic nature of the soil forming processes. For purposes of orientation the exchange capacity value of C might be used as the starting point to bring out the facts about the comparative rôle of the organic and mineral fractions in the base exchange reactions of the soil. The figures in table 1 reveal that the seat of the base-exchange capacity in A_0 lies primarily in the organic fraction.

Mattson (3) prepared by the Odén method a sample of "humic acid" which he found to have an exchange capacity of 306.4 m. e. per 100 gm. This would seem to indicate that the organic matter of the A_0 layer does not contain much of this humic acid. Analyses of 12 A_0 samples (only 4 are given in table 1) have given only one case where the base exchange capacity was equal to 37.2 m.e. per 100 gm. Thus, at best, the organic matter would contain about 10 per cent of the "humic acid." We know, however, that the humic acid is not the only organic constituent capable of base exchange. It may, therefore, be inferred that the state of decomposition which is responsible for the formation of the many organic complexes in A_0 will influence the base-exchange capacity. In table 2, reproduced from Muller (5), figures are presented which show that the stage of decomposition does influence the base-exchange capacity of organic materials.

TABLE 2

Base exchange in decomposing organic materials

TIME	BASE EXCHANGE PER GRAM DRY WEIGHT		
	Soybeans	Straw	Manure
	m.e.	*m.e.*	*m.e.*
Beginning	0.58	0.00	0.73
After 3 weeks	....	0.22	1.03
After 2 months	0.52	0.30	1.09
After 3.5 months	0.48	0.33	1.11
After 6 months	1.20	0.38	1.45
After 9 months	....	0.59	1.67

Unsaturation of A_0

Table 1 also brings out the next important chemical property of the A_0 layer in podzols, its unsaturation. It varies from 5.3 to 90 per cent. This wide variation is due primarily to two factors: parent material and vegetation.

Parent material as a factor of unsaturation

In the podzol process of soil formation the mineralized bases from the A_0 layer go downward to be partially "filtered" out, or adsorbed, by the B horizon. There they are caught by the plant roots and, together with the bases which are picked up by other roots in the soil solution of the intermediate layers—between A_0 and B—, they are transferred to the tops of the plant cover. They appear again at the surface when the leaves, twigs, and branches die and are redeposited in the A_0 layer to be mineralized. The richer the parent material the greater the circulation of the bases; the state of unsaturation will therefore depend on the balance between the outgo and return of the bases.

Because of that, soils which have a parent material rich in bases will resist podzolization. This seems to be the case with the Washington loam as shown in table 1. Its parent material consists of glacial drift which contains some limestone. Naturally more calcium is pumped up from the lower horizons and redistributed in A_0. This is reflected in the reaction of the material. A water extract of it shows a pH of 7.2 and even a neutral salt extract shows only slight acidity—pH 6.8.

Ammonification and unsaturation

This type of unsaturation is not to be confused with a temporary condition of a similar nature—increase in alkalinity—because of the formation of ammonia in the process of ammonification. It is well known that the base exchange complex of the podzols, especially the A_0 layer, may contain some ammonia. The little work done by the author indicates that it varies seasonally and in the late spring ammonia may be detected frequently. Undoubtedly it is linked up with the relative supply of carbohydrates and nitrogenous substances. This problem needs to be investigated further.

Vegetation as a factor of unsaturation

The wide variations in the ash content of plants contribute a good deal to the speed of podzolization. Unfortunately not very many analyses on the forest litter are available. It is known, however, that as a rule the hardwoods contain a higher ash content than the conifers.

In table 3 ash analyses and CaO content of wood, bark, and forest litter of a number of hardwoods and conifers are given.

A comparison of the average ash content of the forest litter in the conifers[2] with the average ash content of the litter in hardwood shows that the hardwoods bring into circulation more ash than the conifers. *The average ash content of the conifers is equal to 2.54 per cent* with a minimum of 1.29 and a maximum of 3.31 per cent, whereas *the average of the hardwoods is equal to 6.63 per cent* with a minimum of 3.85 per cent and a maximum of 11.74 per cent. There is 2.6 times as much ash furnished by the hardwood litter as by the conifer litter. Even the wood and bark of the hardwoods has a higher ash content than the wood or bark of the conifers, as shown in table 3.

Another point to be considered in this connection is the rate of decomposition of the litter. Hardwood litter decomposes much faster and more completely than conifer litter. There are fewer intermediary split products of decomposition under conditions of hardwood litter than under conifer litter. Thus the final products of mineralization are more active in the hardwood

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[2] The average for the conifers was obtained by adding the ash percentages from No. 1 to 10 in table 3 and dividing by 8, the number for which analyses are shown. Similarly the average ash content of the hardwoods was calculated.

TABLE 3

Ash constituents of some forest trees

NUMBER	SPECIES	ASH CONTENT IN			CaO CONTENT IN	
		Wood	Bark	Forest litter (old leaves)	Forest litter	Wood
		per cent	*per cent*	*per cent*	*per cent*	*per cent*
1	Yellow pine (*Pinus mitus*)†	0.3765	.....	.....	....	0.163
2	Old field pine (*Pinus mitus*)†	0.2620	1.81	.....	....	0.113
3	Red pine (*Pinus resinosa*)‖	......	.....	2.30	....	.....
4	White pine (*Pinus strobus*)‖	......	.....	2.70	....	.....
5	White pine (*Pinus strobus*)**	......	.....	2.90*	....	.....
6	Pine (*Pinus sylvestris*)§	......	.....	1.46	0.59	.....
7	Pitch pine (*Pinus rigida*)**	......	.....	1.29	....	.....
8	Black pine (*Picea nigra*)†	0.2874	.....	.....	....	0.123
9	Red spruce (*Picea rubra*)‖	......	.....	3.30	....	.....
10	Red spruce (*Picea rubra*)**	......	.....	3.31	....	.....
11	Balsam fir (*Abies balsamea*)**	.....	.....	3.08	....	.....
12	Larch (*Larix europaea*)**	.....	.....	6.09	....	.. ..
13	Red maple (*Acer rubrum*)‖	0.2714†	3.48†	4.40	....	.....
14	Red maple (*Acer rubrum*)**	......	.....	4.08	....	.. ..
15	Sugar maple (*Acer saccharum*)‖	......	.....	11.30	....	.. ..
16	Sugar Maple (*Acer saccharum*)**	......	.....	6.68	....	.....
17	Black locust‡	......	.....	11.74	....	.....
18	Birch‡	......	.....	4.68	....	.....
19	Grey birch (*Betula populifolia*)‖	......	.....	4.10	....	.....
20	Grey birch (*Betula populifolia*)**	......	.....	4.07	....	.....
21	Beech (*Fagus grandifolia*)**	......	.....	4.49	....	.....
22	Beech (*Fagus grandifolia*)**	......	.....	9.26	....	.....
23	Beech (*Fagus grandifolia*)**	......	.....	9.36	....	.....
24	Beech (*Fagus grandifolia*)**	......	.....	7.11	....	.. ..
25	Beech (*Fagus grandifolia*)§	......	.....	5.57	2.46	.....
26	Bird cherry‡	......	.....	7.24	....	.....
27	Chestnut (*Castanea vulgaris*)†	0.1500	3.43	4.55‡	....	0.050
28	Hickory (*Carya tomentosa*)†	0.6468	3.86	.....	....	0.161
29	Redoak (*Quercus rubrum*)†	0.7687	6.28	.....	....	0.246
30	White oak (*Quercus alba*)†	0.3927	5.88	.....	....	0 083
31	Post oak (*Quercus obtusiloba*)†	0.9224	11.80	....	....	0.301
32	Dogwood (*Cornus Florida*)†	0.8029	9.62	....	....	0.223
33	Ash (*Fraxinus americana*)†	0.4247	.....	10.26**	....	0.075
34	Poplar (*Populus grandidentata*)**	......	.....	3.85	....	.....
35	Sycamore (*Plantanus occidentalis*)†	1.00	.....	.....	....	.....

* Average of three lots.

† Results taken from White (9).

‡ Results taken from Tollens (7).

§ Results taken from Glinka (1).

‖ Results taken from C. W. Watson, personal communication from unpublished data.

** Results taken from Melin (4).

forests, and, being basic in reaction, they contribute a great deal to the retardation of the podzolization process in hardwood forests. All in all, the hardwood vegetation, furnishing a higher ash and consequently higher base content, will help to keep the unsaturation of A_0 at a low level. This in turn will retard the process of podzolization.

The case of the brown soils ("Braunerde" of Ramann) which are climatogenically in the podzol zone and still not thoroughly podzolized has been discussed elsewhere (2). It is the parent material and the vegetation that enter into the picture of the process of forming the "Braunerde."

THE A_1 HORIZON

Morphological[3] *and chemical characteristics*

In a true podzol the A_1 horizon is the mineral horizon lying immediately below the A_0 layer. On a loamy parent material its general morphological features are as follows: It is light gray with a straw-colored tinge, impregnated with some humus material, and is of a powdery, loose, silty structure, with a sandy appearance and fine porosity. As a matter of fact it has no structure and this structureless condition is one of the distinctive characteristics of this horizon. The texture is not sandy either, rather it is loamy. In thickness it varies, but on the average it is about 14 cm.

Chemically A_1 is distinguished by its low base exchange·capacity—as compared with A_0—loss of bases and sesquioxides, enrichment in SiO_2, and a comparatively high unsaturation.;

In a medium podzolized soil where the morphological attributes are not in evidence the chemical properties determine the degree of podzolization.

A morphological study made on the profiles of the loam soils mentioned in table 1 reveals no apparent features of a podzol. The A_1 horizon instead of being light gray is yellowish brown in the Sassafras loam; reddish brown to brown in the Washington loam; darker reddish brown in the chestnut, which contains a considerable amount of organic matter; and brownish red in the Penn silt loam.[4] Thus the A_1 horizons in the soils mentioned do not exhibit the morphological features which correspond to those in a true podzol loam.

When we come to examine the chemical properties of the A_1 horizons in the respective soils as presented in table 4, we note that these do correspond, or at least show tendencies to correspond to the chemical attributes of the loam podzols.

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[3] There are some physical properties of the soil, such as structure, and porosity, which are easily detected by morphological methods and are therefore considered together with the morphological characteristics of the soil.

[4] The Penn series of soils seem to belong to what Glinka (1) calls "endodynamomorphic soils in which the factor of the parent material predominates over the climatic factor for a long time in the process of soil formation."

Base exchange capacity and unsaturation

In table 5 a comparison is made on the base exchange capacity and unsaturation of the A_0, A_1, and C horizons.

TABLE 4

Total chemical analyses of soil profiles

LABORATORY NUMBER	HORIZON	DEPTH OF HORIZON	REACTION		SiO_2	Fe_2O_3	Al_2O_3	P_2O_5	CaO	MgO	Mn_3O_4	BASE EXCHANGE CAPACITY	UNSATURATION (H)	
			H_2O extract	Neutral salt extract										
		cm.	*pH*	*pH*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*m.e.*	*m.e.*	*per cent*
						Sassafras loam								
1	A_0	3–4	5.0	3.8	76.05	3.127	5.322	0.123	0.347	0.472	.....	22.30	19.70	88.3
2	A_1	15	5.2	4.4	85.33	4.128	5.086	0.080	0.247	0.479	0.144	5.46	5.14	94.1
3	A_2	18	5.0	4 4	85.78	4.575	8.562	0.085	0.456	0.611	0.312	4.74	4.27	93.6
4	B	14	5.1	4.4	84.35	4.575	6.23	0.101	0.513	0.629	0.225	4.98	4.05	81.3
5	C	..	4.9	4.5	84.30	4.743	6.75	0.090	0.339	0.679	0.157	5.2	4.05	78.0
						Penn silt loam								
7	A_0	3–4	5.2	4.3	52.55	6.08	17.06	0.131	0.744	1.729	0.445	26.4	18.64	70.6
8	A_1	12	4.9	4.5	57.85	8.15	18.09	0.071	0.304	1.743	0.487	14.1	7.56	53.6
9	A_2	26	4.8	4.5	56.7	8.53	19.53	0.056	0.342	1.993	0.238	10.7	7.56	70.6
10	B	38	4.9	4.3	54.97	9.54	20.36	0.0467	0.206	2.166	0.425	11.4	7.56	66.3
11	C	..	5.1	4.3	55.10	9.21	20.20	0.055	0.206	2.053	0.437	11.2	7.64	68.2
						Chester gravelly loam								
22	A_0	3–4	6.2	5.0	63.20	5.44	10.905	0.127	0.831	0.604	0.200	18.5	5.83	31.5
23	A_1	12	5.5	4.4	71.45	6.418	10.717	0.092	0.693	0.669	0.281	7.19	4.568	63.5
24	A_2	16	5.4	4.4	72.55	7.142	9.877	0.097	0.736	0.773	0.275	6.31	3.46	54.8
25	B_1	15	5.2	4.4	68.37	9.290	10.565	0.102	0.583	0.769	0.344	6.8	4.49	66.0
26	B_2	43	5.2	4.4	63.25	14.575	11.253	0.108	0.363	0.263	0.294	6.61	3.34	50.5
27	C	..	5.4	4.6	68.92	7.54	11.002	0.066	0.565	0.301	0.338	5.3	2.38	45.0
						Washington loam								
17	A_0	3–5	7.2	6.8	61.40	5.86	10.115	0.175	1.063	0.778	0.325	37.20	1.98	5.3
18	A_1	21	5.3	4.4	71.57	6.64	11.297	0.114	0.355	0.654	0.255	12.00	5.97	50.0
19	A_2	11	5.1	4.4	69.12	7.73	12.74	0.131	0.286	0.636	0.275	8.16	5.37	65.8
20	B	19	5.3	4.4	65.55	8.43	13.52	0.132	0.215	0.579	0.275	6.24	3.19	51.1
21	C	..	5.4	4.8	66.3	8.567	12.93	0.128	0.237	0.692	0.183	5.03	2.2	43.7

It is striking what a drop in base exchange capacity takes place from A_0 to A_1. Undoubtedly this drop may be explained by the difference in the organic matter content of A_0 and A_1. With the tremendous base exchange capacity inherent in the humus complexes, it may be inferred that the mineral horizon A_1 with its low base exchange capacity is poor in organic matter. If we examine the data in table 5 a little closer, we see that the base exchange capacity of A_1 in the Sassafras soil corresponds to that of C, the parent material, which is supposed to contain only traces of organic matter. Looking at it from this angle, one would be apt to surmise that in the Sassafras soil the base exchange capacity of A_1 is due to the mineral complexes. But we must not forget that A_1 is the horizon of eluviation where the leaching processes are instrumental in decomposing the mineral complexes, in breaking up the kaolin nuclei, leaving behind SiO_2. All of that contributes to a decrease of the base exchange capacity of the mineral fraction especially in an acid medium as the case is with the A_1, which is brought out by the pH values. It must therefore be concluded that some of the base exchange capacity in A_1 is to be attributed to organic matter.

TABLE 5

Base exchange and acidity in A_0, A_1, and C

SOIL SERIES AND HORIZONS	REACTIONS		BASE EXCHANGE CAPACITY	H	UNSATURATION
	H_2O extract	Neutral salt extract			
	pH	*pH*	*m.e.*	*m.e.*	*per cent*
Sassafras loam A_0	5.0	3.8	22.3	19.7	88.30
Sassafras loam A_1	5.2	4.4	5.46	5.14	94.10
Sassafras loam C	4.9	4.5	5.20	4.05	78.00
Penn slit loam A_0	5.2	4.3	26.40	18.64	70.60
Penn slit loam A_1	4.9	4.5	14.10	7.56	53.60
Penn slit loam C	5.1	4.3	11.20	7.60	68.20
Chester gravelly loam A_0	6.2	5.0	18.50	5.83	31.50
Chester gravelly loam A_1	5.5	4.4	7.13	4.57	64.00
Chester gravelly loam C	5.4	4.6	5.30	2.38	45.00
Washington loam A_0	7.2	6.8	37.20	1.98	5.30
Washington loam A_1	5.3	4.4	12.00	5.97	50.00
Washington loam C	5.4	4.8	5.03	2.20	43.70

An examination of the data in table 5 on the other soil series shows that A_1 has a greater base exchange capacity than C. In the Washington loam it is almost 2.5 times as high. This indicates a high organic matter content. Thus a comparison of the base exchange capacity of A_1 (for that matter any other horizon) and C may serve as an index of the organic matter content of the respective horizons. This problem is under investigation and in due time the results will be reported.

The high unsaturation of A_1 in the four series reported in table 5 is ample chemical evidence of the podzolized state of these soils. In all soils, except the Penn series, the percentage of unsaturation of A_1 is higher than that of A_0 and C. The Penn series of soils does not behave as a climatogenic[5] soil: its characteristic features were formed not so much because of the climate and vegetation factors but because of the specific nature of the parent material. According to Zakharov (11) such a soil is to be called "lithogenic" instead of "climatogenic." And even in this soil one may find chemical evidence of podzoliation, as will be pointed out presently.

Silica

Coming back to table 4, which gives the inorganic constituents of the soils under discussion, we may note that in all cases the SiO_2 is higher in A_1 than in any other horizon except A_2. In a true podzol the difference in SiO_2 content of A_1 and A_2 is more marked than in podzolized soils—those discussed in this paper.

The fundamental reactions involved in enriching the A_1 horizon with SiO_2 in the podzol process of soil formation are as follows: The A_1 horizon is subjected to the action of the carbonic acid formed by the respiration of roots and metabolic activities of microorganisms in decomposing organic materials, nitric acid from the processes of nitrification, sulfuric acid from the processes of oxidation of sulfur compounds, other inorganic acids like phosphoric and silicic in various forms, and finally the organic acids from the decomposition of organic matter. All of these acids which form both in the A_0 layer overlying the A_1 horizon and in A_1 itself react with the minerals, decompose them, set free first of all the alkali and alkaline earth bases, then split the kaolin nuclei, setting free the sesquioxides and leaving behind the SiO_2.

Calcium and magnesium

A low content of alkaline earth bases in A_1 in a soil with a relatively higher content of these in C is indicative of strong podzolization. These two bases, especially the Ca, circulate through the soil profile more than any of the other cations. They return in appreciable quantities to the A_0 through the medium of the roots and plant residues which are deposited at the surface as outlined previously. The data in table 4 on the Ca and Mg content of A_0 show clearly that it is the organic matter which mobilizes the bases.

In the soils under consideration the Ca and Mg content is relatively high, even higher than in the C horizon—the parent material. From the data available on the chemical composition of loamy podzols it is known that the Ca and Mg content is rarely higher in the A_1 horizon than in the C. These soils are therefore not true podzols since they show consistently high Ca and Mg in A_1 and even in A_2. It is well to remember that in true loamy podzols the Ca and Mg is frequently higher in A_1 than in A_2 just because of the higher organic matter content in this horizon.

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[5] More about the climatogenic soils is to be found in a previous publication (2).

Iron and aluminum

None of the chemical constituents of the soil material in the profile of the podzols are more indicative of the state of podzolization than the sesquioxides. In the circulation of the elements through the profile, the sesquioxides are among those which do not return in any appreciable quantities as the bases do. They are always lower in the A_1 horizon than in the parent material. And the greater the difference between A_1 (and more so in A_2, as we shall see) and C, the greater is the degree of podzolization.

In the podzol zone the conditions inducive to the movement of the sesquioxides are a high hydrogen-ion concentration, depletion of electrolytes, especially bases and anions with which the sesquioxides combine, or in other words, the conditions which keep the sesquioxides below the isoelectric point of precipitation. These conditions prevail in A, the horizon of eluviation, and A_1 as a component of A reflects these conditions by becoming impoverished of the sesquioxides. This is true for podzols with a mature profile. In the soils under consideration, as shown in table 4, the sesquioxides in A_1 are lower than in C, a true indication of the podzolized condition of these soils, but the difference between the two horizons is not great enough to place these soils in the strongly podzolized class.

An important characteristic of the A_1 horizon is its power to fix humates with the alkaline earth bases and sesquioxides which originate in the A_0 layer in the process of decomposition and mineralization of the organic matter. There is no accumulation of these in A_1 since they are also subject to decomposition. But in the circulation of the soil constituents these newly formed substances are temporarily retained in the A_1 horizon. The free acids and acid salts that pass beyond A_1 react with the mineral constituents of the underlying horizon which, because of that, is leached more than the overlying A_1 horizon.

THE A_2 HORIZON

Morphological and chemical characteristics

In a podzol soil of a loamy texture the A_2 horizon is a component part of the horizon of eluviation. It differs from the overlying A_1 horizon in being more thoroughly podzolized. Its morphological features are: The color is bleached ash-gray to whitish gray changing to a brownish tinge at the bottom. The average thickness of this horizon in a loamy soil is 14 to 16 cm. Structurally this horizon varies little from the horizon above, being loose and powdery when dry, smeary when wet, with little pore space, blending into a platy structure at the junction with the underlying horizon, sometimes with granules of ortstein or concretions.

None of the morphological features of the A_2 horizon are more pronounced than the bleached ash-gray color. It is this striking color that makes horizon A_2 the outstanding characteristic horizon in the podzol profile. As to the source of the color there seems to be a general agreement that it is due to the SiO_2 which separates out from the decomposed kaolin and coats the soil particles. This process, as pointed out in the discussion on the SiO_2 content in the A_1 horizon, is intimately connected with the activity of the reaction products of the A_0 layer. But in A_1 the color is less pronounced because the humus complexes, being dark in color, obscure the color effects of the white SiO_2. In the A_2 horizon the prevailing organic matter complexes belong to the colorless "crenic" acid type as pointed out by Williams (10).

Chemically A_2 may be looked upon as an accentuated condition of A_1. It is lower in base exchange capacity than A_1, more unsaturated, usually lower in Ca and sesquioxides and higher in SiO_2. In a true podzol loamy soil the SiO_2 penetrates in tongue-like fashion into the underlying horizon B as shown in plate 1 reproduced from Zakharov (11).

As in A_1 there is no evidence of the characteristic morphological features of a podzol in the A_2 horizon of the soils investigated. It is true that the A_2 is slightly lighter in color than A_1, but it is due not to the SiO_2 coating, but to the lower humus content.

An analysis of the chemical data on the constituents of A_2 as given in table 4 shows that in some respects this horizon exhibits the chemical properties of podzolized soils.

Base exchange capacity and unsaturation

A graphic illustration of the tendencies of the base exchange capacity of the soils investigated as compared with that of a true podzol on a heavy loam as reported by Rodé (6) is given in figure 1. It will be noted that the base exchange capacity of this horizon is lower than that of A_1, which is in agreement with the general chemical features of a podzol. The tendency of the curves of the soils investigated is to follow the curve of the true podzol up to A_2. After that the curves depart from the tendency of the curve of the true podzol. This shows that the soils are podzolized to some degree, but that they are not true podzols.

The percentage of unsaturation of A_2 in two of the soil series—Chester and Washington—runs true to form as in podzols, that is, it is higher in this horizon than in any other. It is also high in the Sassafras series, but not higher than A_1, but higher than in any of the other horizons. In the Penn series the same tendency may be noted, even though it is not a true climatogenic soil. Thus the unsaturation of A_2 furnishes chemical evidence of the podzolized state of the soils investigated.

At this point it is worthy of note that the pH of the water extract of the soil material from A_2 is as a rule lower than in any other horizon. The highly unsaturated condition of this horizon is suggestive in this connection. The total acidity as measured by the milligram equivalents of hydrogen is low in A_2 when compared with the other horizons. This indicates a low buffer capacity.

SiO_2

The figures in table 4 show that in comparison with the C horizon there is an accumulation of silica in the A_2 horizon. In the Chester and Sassafras series the SiO_2 content is higher than in any other horizon. This property is true for genuine podzols, except that it is more sharply defined, i.e. the difference between the SiO_2 content of the A_2 and any other horizon is greater than in the soils under consideration.

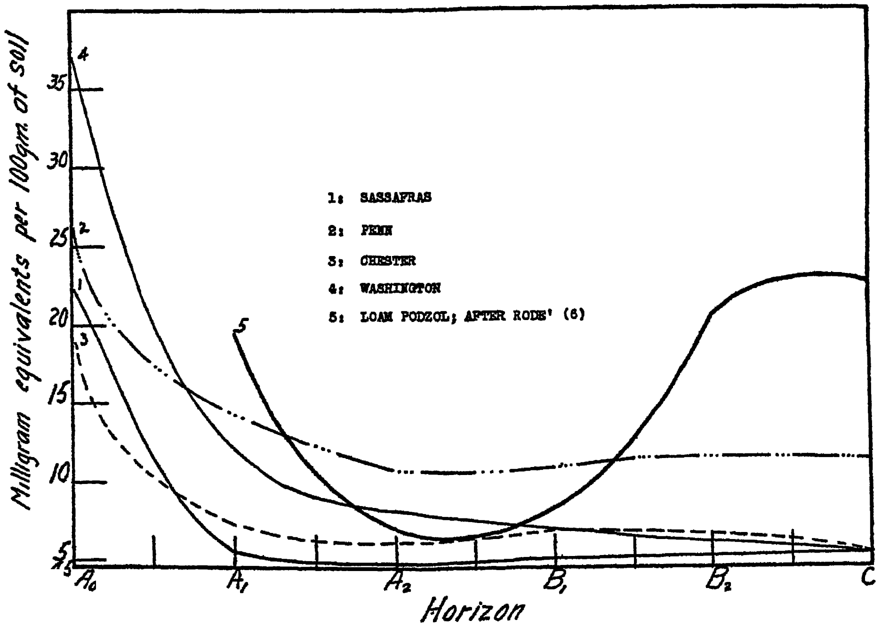

FIG. 1. BASE EXCHANGE CAPACITY OF SASSAFRAS, PENN, CHESTER, AND WASHINGTON SOILS, AND OF A LOAM PODZOL

Calcium and magnesium

What has been said about the bases Ca and Mg in connection with the description of A_1 holds true also for A_2. As long as A_2 is not completely podzolized—and hence leached—the base content will depend on the amount supplied by the A_0 horizon. From the data in table 4 it is clear that the Ca and Mg are high in A_2 (even higher than in A_1, except in the Washington series), a property contrary to the podzolized state of A_2 in a true podzol. It is an indication of the weakly podzolized condition of the soils investigated.

Iron and aluminum

The data in table 4 on the sesquioxide content of A_2 do not bring out clearly the fact that these soils are podzolized. Instead of being lower than in A_1

the sesquioxides are higher. And yet they are lower than in C—the parent material. From what has been said about the circulation of the sesquioxides in the soil profile (see section: "The A_1 horizon," iron and aluminum), the comparative values for A_2 and C do indicate a certain degree of podzolization.

THE B HORIZON

Morphological and chemical characteristics

The distinctive feature of the B horizon is that it is accumulative in character. It is known as the horizon of illuvation (washing in) in contrast to the A horizon known as the horizon of eluviation (washing out). The soil constituents which leach out from A are partly retained by the B horizon. This horizon is also enriched with the fine particles—mineral and organic—from the overlying A horizon by mechanical movement. It seems as if the B horizon were destined by the natural forces of soil formation to protect the soil body from being destroyed. By virtue of its accumulative properties this horizon becomes the storehouse from which the plant roots extract mineral nutrients and send them to the stems and leaves, which furnish the material for the A_0 layer—the starting point of the circulatory system of minerals and other substances in the process of soil formation. All of these facts leave a definite impression on the morphological, physical, and chemical features of this horizon.

In a loam podzol the accumulative horizon is divided into two subhorizons: B_1 and B_2 which are recognized as independent horizons because of variations in their morphological, physical, and chemical properties.

In plate 1 the B_1 and B_2 horizons are distinctly featured.

B_1 is usually 30–40 cm. deep, of a dull brownish coloration with some light and some dark streaks or veins, pocket-like on top and extending deeper in tongue-shaped fashion. The light colored markings are due to the accumulation of some of the podzolized material which moved into this horizon from the overlying A_2 horizon. The dark streaks represent the humus materials from A_0 and A_1 which pass in the sol state through A_2 and precipitate with the sols of iron, aluminum, and manganese by virtue of the higher concentration of electrolytes in this horizon and probably also as a result of the mutual coagulation effects of the oppositely charged colloids. (The SiO_2 and humus are negatively charged, whereas the iron and aluminum are, at the reaction existing in this horizon, positively charged.)

Because of the infiltration of materials from above, the B horizon is compact and heavy. It attains a more loamy texture, becomes sticky, and at the top begins to show signs of laminated or platy structure. With depth this structure becomes more apparent and toward the bottom this horizon exhibits a nutty and prismatic structure.

B_2 begins just below the prismatic structure and extends to a depth of about 30 to 40 cm. As a part of the accumulative horizon B_2 has properties similar to B_1, but it is poorer in organic matter and perhaps for that reason it is of a lighter brownish coloration. It has fewer if any veins of the bleached material from A_2. At the bottom this horizon becomes lighter in texture, is not so compact and blends in with horizon C, the parent material.

It is to be noted that both B_1 and B_2 have the coatings of SiO_2, humus, manganese, and sesquioxides on the faces of the structural units, along the root paths and cracks. Infrequently, especially in lighter soils, orterde, ortstein, and concretions form along these cracks.

Chemically the B horizon is distinguished by the lower (when compared with the other horizons) SiO_2 content, high sesquioxide content, increase in bases and in capacity for base-exchange, and lower unsaturation (when compared with A_2). B_1, as a rule, has a higher organic matter content than A_2.

In the data in table 4, one might find, on the one hand, enough evidence of genuine podzol properties in the soils investigated and, on the other hand, chemical characteristics which show no, or only slight, podzolization. From the standpoint of morphological attributes one may note that only in one case—the Chester series—was it possible to differentiate two horizons (B_1 and B_2) in the horizon of accumulation, which indicates an immature profile; hence these soils are not true podzols.

Base exchange capacity and unsaturation

In the soils investigated the base exchange capacity of the B horizon has increased as compared with A_2, showing that some of the colloidal substances from the overlying horizons did move downward and stopped at the B horizon. It is to be remembered that the movement of the colloids, in which by the way, the base exchange capacity is high, is a true indication of the podzol zonal type of soil formation. The tendencies of the base exchange capacity are brought out with more clarity in figure 1. The base exchange capacity is high in A_0, decreasing in A_1 and continuing to decrease in A_2, and increasing again in B. The base exchange capacity of B is as a rule higher than that of C, except where that of the parent material is originally very high, as in the soil represented by curve 5, which has varved clay as the parent material.

True to form, the percentage of unsaturation in B is lower than that in any of the other horizons. This would indicate that the alkaline earth bases and probably potassium are present in the exchange complex.

SiO_2

Since very small quantities of the silicates are being attacked in the B horizon there is no free SiO_2 accumulating in it aside from the amorphous SiO_2 from the overlying horizons. Besides, it is enriched with iron, aluminum, and other colloidal complexes. All of that is inducive to a relative lowering of the total SiO_2. The data in table 4 on the SiO_2 content of the B horizon show this to be the case with the soils investigated.

Calcium and magnesium

In a true podzol the content of alkaline earth bases increases in the B horizon. As the humus sols move through the horizon of eluviation in an acid medium and reach the B horizon which is less acid, they precipitate and probably fix the alkaline earth bases and sesquioxides. It is known from the work of Mattson (3) that the colloidal humus complexes have a definite isoelectric point of precipitation which depends on the pH of the dispersion medium and on the presence of electrolytes. In podzol soils the conditions in the B horizon seem to be favorable—probably the proper pH and electrolyte content—for isoelectric precipitation of the humus sols with the bases and sesquioxides. That this condition has not been reached in the soils investigated may be judged from the data in table 4 on the Ca and Mg content of the B horizon. It is lower than in A_1 or even A_2, which shows that these soils are only in the early stages of podzolization. Whether they will ever become thoroughly podzolized is another question which needs further investigation. As it is, the alkaline earth bases in these soils seem to become tied up and fixed in the horizon of eluviation, as pointed out in the discussion on the A_1 horizon.

Iron and aluminum

It has been mentioned that the translocation and distribution of sesquioxides in the soil profile may serve as one of the best attributes of the state of podzolization of the soil. Very little of the iron and still less of the aluminum is returned to the A_0 horizon to go again into circulation through the soil body. As a result of that the sesquioxides accumulate in the B horizon and, even in the early stages of podzolization, an increase in these may be noted. They may be found in combination with the humus or as gels which infrequently are transformed into definite minerals such as gibbsite and limonite. They serve as cementing materials in the process of orterde or orstein formation. The work of Lundblad to which reference has been made (2) shows that in the B horizon of podzols there is a higher amount of gel than in any other horizon. This has also been proved earlier by Gemmerling, whose work has been quoted in an earlier paper (2).

From the data in table 4 on the sesquioxide content of the soils investigated, it is clear that we are dealing with distinctly podzolic soils, but the accumulation does not warrant their classification with the true podzols.

Phosphorus and manganese in the profile

No significant facts are brought out by the data on the Mn content of the various horizons. As a negative colloid Mn should undergo the reactions similar to humus, which is also negatively charged, provided both of them have the same isoelectric point of precipitation. It has been shown by Udulft (8) that these two colloids behave alike toward carbonates and bicarbonates.

Phosphorus is one of the very immobile anions in the soil, but in the podzols there is usually an accumulation in the A_0 and B horizons. In the former it apparently exists in the organic forms whereas in the latter it is fixed by the

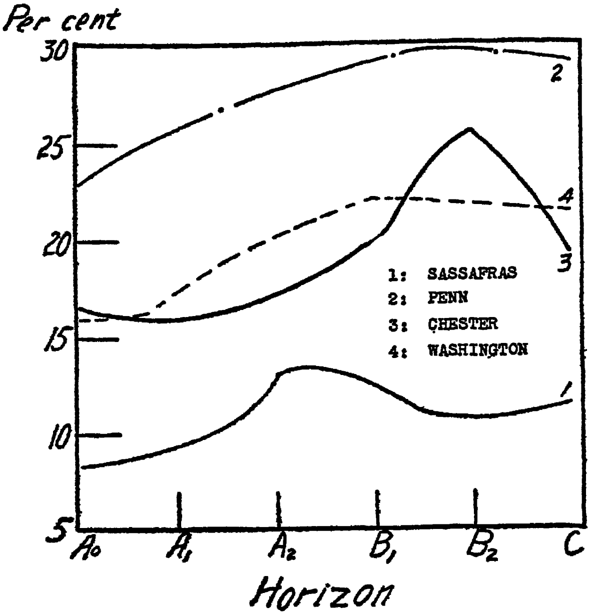

FIG. 2. SESQUIOXIDES OF SASSAFRAS, PENN, CHESTER, AND WASHINGTON SOILS

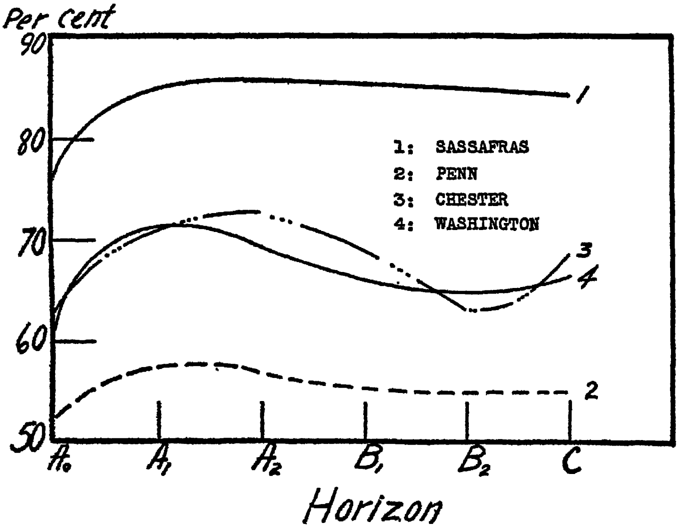

FIG. 3. SiO_2 OF SASSAFRAS, PENN, CHESTER, AND WASHINGTON SOILS

bases and sesquioxides. In the soils investigated it may be noted that the P_2O_5 content is invariably high in the A_0, but only indications of accumulation may be noted in the B horizon.

DISCUSSION

A survey of the chemical constituents in the profiles of the soils investigated clearly shows that the more typical reactions which occur in a podzol are to some degree apparent in these soils. Especially is this true for the values of base exchange capacity, sesquioxides, silica, and unsaturation.

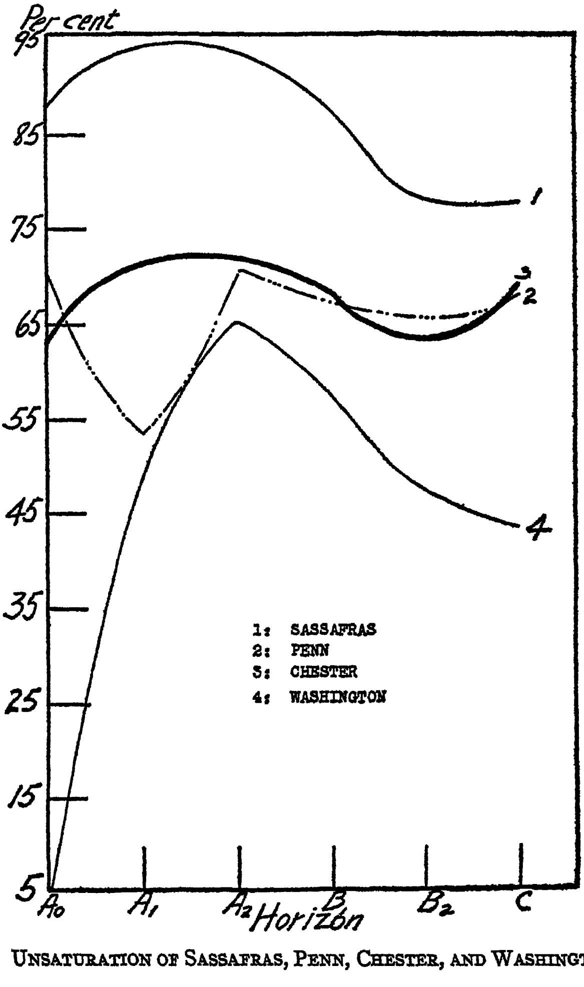

FIG. 4. UNSATURATION OF SASSAFRAS, PENN, CHESTER, AND WASHINGTON SOILS

In genuine podzols the base exchange capacity is high in the A_0, decreases abruptly in A_1, is still lower in A_2, and increases again in the B horizon. This is brought out in figure 1. The accumulation of the colloidal material in the B horizon is responsible for the increase in this property. Curves 1 to 4 show no sharp differences in the base exchange capacity of the horizons in the soils investigated, which is not true, however, for a genuine podzol as shown in curve 5.

This indicates that the soils investigated are weakly podzolized. The sesquioxides are low in the A_0, are not much lower and are sometimes even higher in A_1 and A_2, and are a good deal higher in B. Only in the Chester and Washington soils does this tendency seem to hold, as brought out in figure 2.

In figure 3 the distribution of the SiO_2 in the profiles of the soils is presented. True to form all soils show an increase in SiO_2 in the A_2 horizon and a decrease in the B horizon. Again the Chester and Washington soils indicate a state of higher degree of podzolization than the other two soils. And still there is no visible (morphological) evidence of podzolization.

In figure 4 the percentage of unsaturation in the various horizons of the soil profiles investigated is presented. It will be noted that the general tendency of the property of unsaturation is to be lower in A_0 than in A_1, increasing up to A_2, and decreasing in B. The degree of unsaturation depends primarily on the reserve of bases furnished by A_0 and on the amount of acids persisting in A_0. It is known that the organic acids—as intermediate products—produced in the decomposition of conifer litter are more resistant to complete decomposition (mineralization) than the intermediate products of hardwood or grass litter. One would therefore expect a higher unsaturation in conifer litter than in hardwood litter. These considerations may explain the peculiar behavior of curve 2 (the Penn series). This particular soil is endodynamomorphic in nature and therefore may not, because of that, follow in every detail the properties of a true climatogenic soil; but as far as the unsaturation is concerned, we need not fall back on its endodynamomorphic nature. It might be explained on the basis of the flora on this soil; it sustains a conifer stand (the other soils have the hardwoods) and perhaps the nature of the litter is responsible for the high unsaturation of A_0.

SUMMARY

The morphological and chemical features of the horizons in a typical loam podzol profile have been surveyed, illustrated, and presented.

A discussion of unsaturation as related to parent materials and vegetation with some data on the ash content of a number of species of forest trees brings out the facts about the speed of podzolization under various plant covers.

The soils for which chemical data are presented show evidence of podzolization; this is illustrated by an analysis of the various chemical indexes which serve as criteria for the degree of podzolization.

On the basis of the data in tables and figures a discussion is presented for each horizon individually, covering the properties of unsaturation, base exchange capacity, sesquioxide, SiO_2 and alkaline earth base content.

The distribution of P_2O_5 and manganese through the profile is also discussed on the basis of data presented.

From the data presented it is clear that the soils investigated are podzolized to a certain degree; they are what some like to call "podzolic soils."

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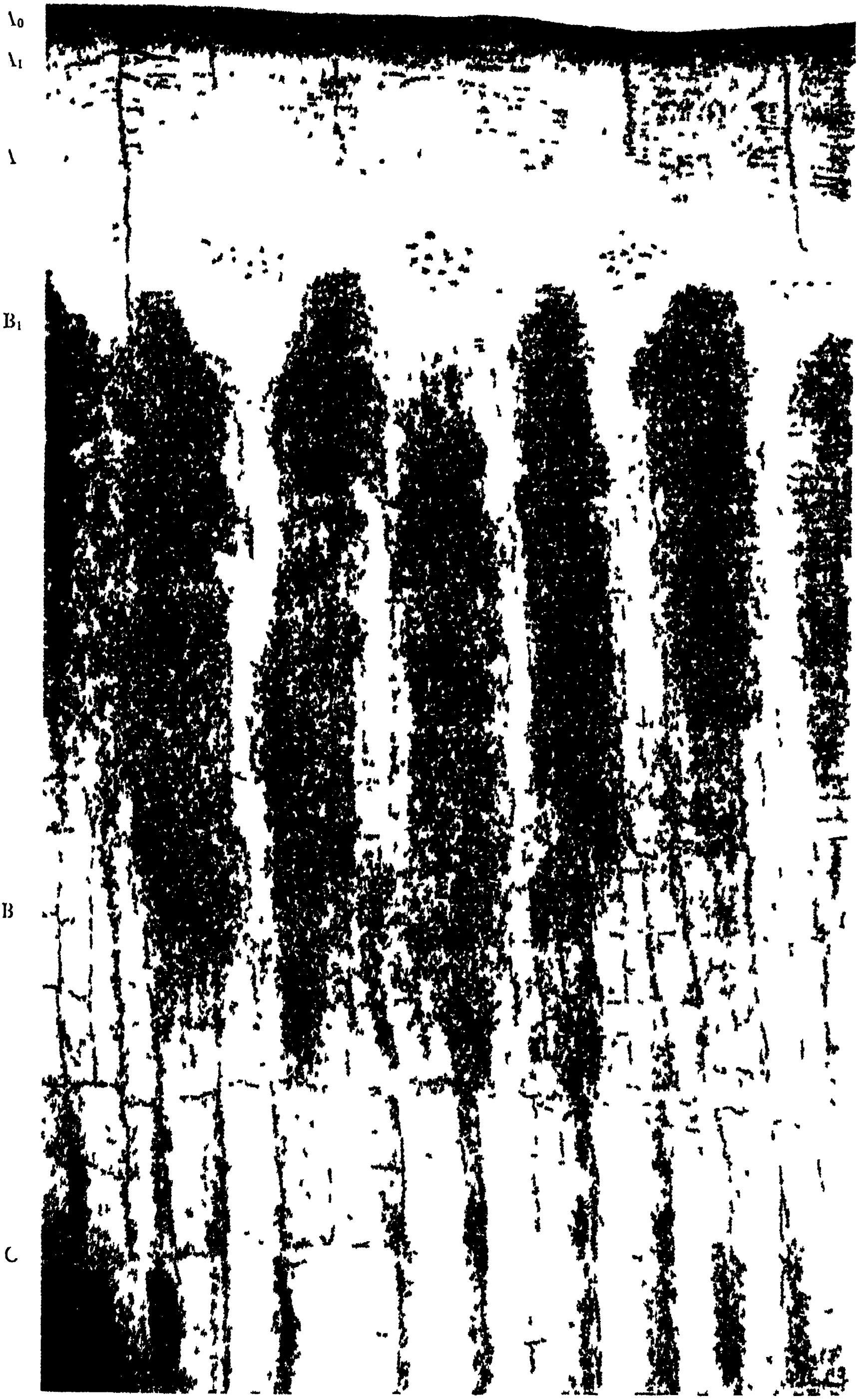
PLATE 1

A Typical Loam Podzol Profile


A0
A1
A
B1
B
C

BOOK REVIEW

Principles of Soil Microbiology, second edition, thoroughly revised. By Selman A. Waksman, Professor of Soil Microbiology, Rutgers University, and Microbiologist of the New Jersey Agricultural Experiment Stations. The Williams & Wilkins Company, Baltimore, 1932. Pp. xxviii + 894, pl. 15, figs. 83.

The subject matter is treated in much the same manner in both the first edition[1] and this second edition. However, many changes have been made in addition to the incorporation of new material which has appeared since the publication of the first edition. In the preface the author states:

> . . . . A number of chapters have been entirely rewritten, especially those dealing with the mycorrhiza fungi and the soil as a medium for plant and animal parasites. A number of new chapters have been added, dealing with the rôle of microörganisms in the decomposition of organic matter in green manures and stable manures, in the formation and decomposition of peat and forest soils, and with the relation between plant growth and the activities of microörganisms in soil. To avoid any considerable increase in the size of the book a certain amount of condensation became necessary so as to balance the added material. This was accomplished by leaving out some of the text which did not bear directly upon the subject under consideration. Several chapters have been combined so as to avoid unnecessary duplication. . . . .

It is unfortunate that, in order to conserve space, it was also necessary to leave out the titles of papers in the references.

The following indicates the nature of some of the changes:

	FIRST EDITION	SECOND EDITION
Total pages	xxviii + 897	xxviii + 894
Text pages	843	829
Chapters	32	34
Chapters eliminated or combined with other chapters		4
Chapters added by expanding original material		6
Figures	77	83
Tables	119	127
Plates	19	15
Figures in plates	154	152
Books listed for reference	204	216
References in text	3,210	4,248

Although these figures do not indicate the large number of changes in reproductions, tables, and text it is quite apparent from the numbers of references that, even though the two books are practically identical in size, much material has been added in the new edition.

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[1] For review of the first edition see *Soil Sci.* 23 (1927): 494–495.

To those familiar with the first edition it seems sufficient to state that the new edition will be a valuable complement to it.

ROBERT L. STARKEY.

A NEW TYPE OF HYDROMETER FOR THE MECHANICAL ANALYSIS OF SOILS

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Received for publication October 29, 1931

Bouyoucos, in a series of papers (1, 2, 3), has discussed the development of his hydrometer method for the mechanical analysis of soils Apart from the serious criticism to which the method has been subjected by Keen (6), Bouyoucos' own comparison of the results obtained by his method and those by the standard mechanical analysis method, do not show a good agreement (2). Out of the 30 soils examined, only 16 showed a fair agreement in the percentages of silt and clay or colloids. The serious discrepancy in the rest of the soils has been explained by supposing that the standard mechanical analysis method classes fine silt along with coarse silt, and the hydrometer method with the clay. All the same the disagreement is there, and no amount of agrument as to the utility (from the practical point of view) of including fine silt in clay, can alter the fact that the hydrometer method, in its present form, cannot give the mechanical analysis of soils as we know it. It might give a set of values which might prove useful in characterizing the behavior of soils in the field, but, as such, it must be considered as an empirical method for determining one of the many "single value" constants of a soil, and should not be confused with the mechanical analysis, which carries with it a definite physical meaning: i.e., a set of values giving percentages of particles of certain well-defined sizes or settling velocities, and all methods for its determination must give essentially the same results.

The hydrometer method possesses an attractive simplicity, but it lacks the precision of theory and experiment associated with the science of pure physics. The insistance of the author on adhering to a set of arbitrarily fixed conditions, shows by itself that the method possesses no fundamental basis. Nor has its value, even as an empirical method been proved beyond dispute, because it has not been shown to give results comparable with that of any other standard method of mechanical analysis, and unless we are prepared to discard all the mass of data already collected, and accept a new and arbitrary basis for the grouping of soil particles (not according to size), the hydrometer method, as it

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[1] This work was carried out by the help of a grant from the Imperial Council of Agricultural Research, and the writer takes this opportunity of recording his indebtedness to the Council. Acknowledgments are also due to the Department of Agriculture, Punjab, for the loan of apparatus and other facilities for work.

stands, will not commend itself to the majority of soil scientists. In the modified form discussed in this paper, an attempt has been made to produce an instrument of precision, which might possess all the advantages of simplicity, accuracy, and reproducibility, and at the same time should have some fundamental basis for its working, so that the necessity of working with a particular instrument, with rigidly defined specifications, is obviated.

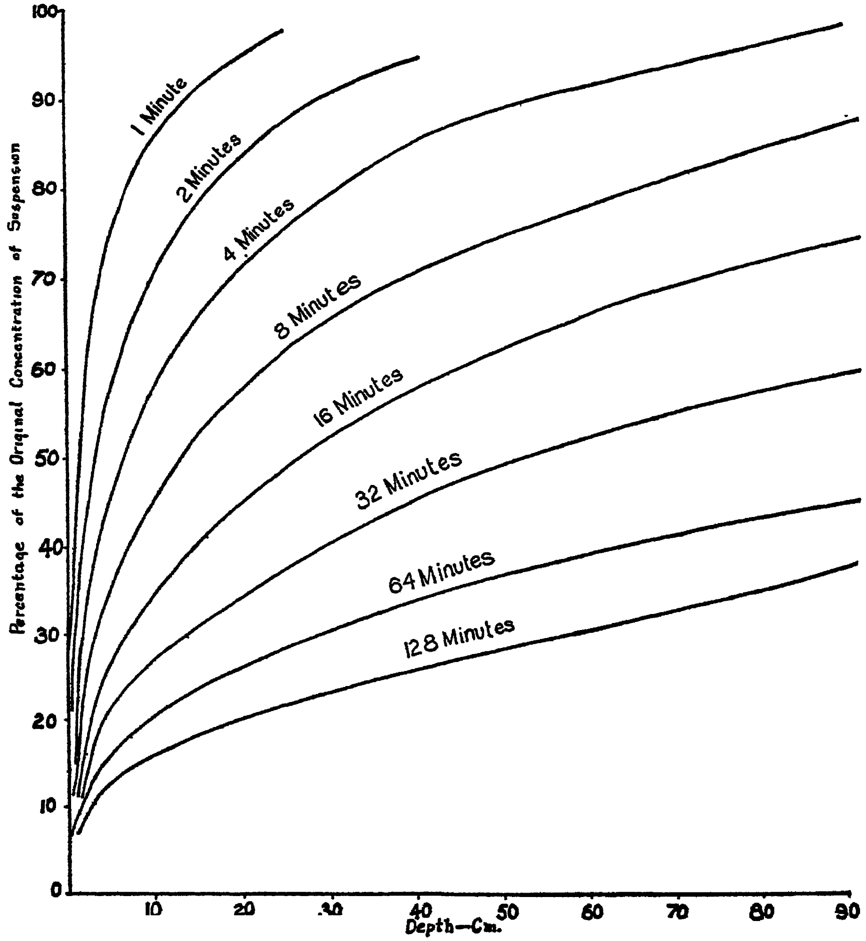

FIG. 1. CURVES SHOWING DENSITY GRADIENT IN A SEDIMENTING COLUMN AT DIFFERENT INTERVALS OF TIME

The successful working of the hydrometer described here, depends upon the fact that the gradient of increasing density, in the suspension of a sedimenting column, becomes less and less steep as we go from top to bottom. In other words, the average density of a column of suspension, the length of which is small compared with the total depth of the sedimenting column, may be taken without serious error as the density of its middle point. This is illustrated in figure 1, in which the density of suspension in a settling column (from top to bottom) at different intervals of time from the beginning of sedimentation, is plotted against depth. The density in every case is expressed as percentage of the original density at the beginning of sedimentation, or the ordinates may be supposed to be the percentages of particles in suspension, the original concentration being taken as 100. The curves refer to soil P.C. 1 (table 1), and have been calculated from the summation curve obtained by the pipette method. It will be seen that there is a rapid decrease in density for the first

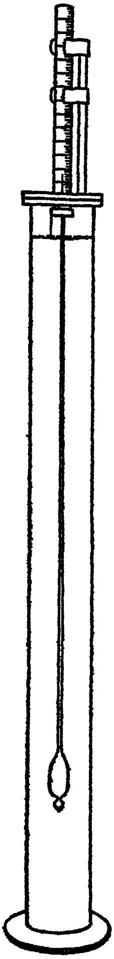

FIG. 2. DIAGRAM OF THE NEW TYPE OF HYDROMETER AND READING DEVICE

20–30 cm., after which the change is comparatively small. The main defect in Bouyoucos' hydrometer is that it records density changes in a region of steep density gradient, and from the nature of the curves in figure 1, the magnitude of error can be comprehended easily.

The new hydrometer shown in figure 2, consists of a wide, but short, bulb (8–10 cm. long) and a thin, long stem (about 0.4 cm. in diameter, and 60–70 cm. long). It therefore, records density changes in a column 8–10 cm. long, and situated at a distance of 50–60 cm. from the top. The settling depth is counted from the top of the sedimenting column to the mean position of the middle point of the hydrometer bulb. Another modification in the hydrometer technique is the arrangement for taking readings. The ordinary method of inserting a paper scale in the stem, is not very accurate, and an error of a few millimeters in the readings might be easily made, as a result of the water surface not providing a sharp line of reference. In this case the tip of an ordinary pin attached to a thin brass cap fitting on the top of the hydrometer stem, forms the reference point, and the readings are taken against the graduations on a burette tube, held in a special clamp. The mouth of the burette tube is closed with a brass cap having a hole in the middle through which the hydrometer stem is passed. The edges of the hole are sharpened to prevent friction that might hinder the free movement of the hydrometer. All that is necessary is to tap the burette tube gently before taking a reading. This brass cap has also a pin attached to it which is required for fixing the position of the burette tube with respect to the water surface. The tube is gradually lowered till the pin point just makes contact with the water surface. The burette tube is held in brass clamps, which keep it in position, at the same time allowing it to be pushed up or down. The brass clamps are fixed to an iron rod attached to an iron cap that can cover the top of the sedimenting cylinder, and has a hole in the middle to allow the free movement of the burette tube. This simple device enables one to take hydrometer readings within a fraction of a millimeter, and does away with the necessity of having a paper scale inside the stem. To avoid parallax the graduations on the burette tube should be all round the circumference as in the Charlottenburg type.

Since the excess of density of the suspension over that of water is proportional to the concentration, direct determinations of the variations with time of the density of the suspension at the mean resting point of the hydrometer would provide data similar in type to those obtained in the pipette method of mechanical analysis and if the foregoing assumption is reasonably correct, the two methods should give closely agreeing values.

EXPERIMENTAL

The most important point to be considered in any method of mechanical analysis is the preliminary treatment to which the soil must be subjected to bring about the maximum dispersion, for anything short of that would be arbitrary and unsatisfactory. Mere shaking, rubbing, or boiling has been proved ineffective; on the other hand, treatment with dilute HCl and H_2O_2 (International method) is too drastic. As the result of a study of factors influencing the dispersion of soil colloids in water (8), the writer has been able to enunciate two broad generalizations:

1. All soils containing only Na as the exchangeable ion (colloids as Na saloid) in the absence of flocculating agents, are completely dispersed on coming in contact with water. They are completely reversible and can be dried and brought into suspension as many times as one likes without any shaking. They show the property of "auto-disintegration," i.e. they would go into suspension as a lump of sugar or salt would go into solution.
2. The foregoing condition is not satisfied until the neutralization of the acidoid is carried to pH 8.5.

These two principles form the basis of the (NaCl-NaOH) method developed by the author and discussed elsewhere (7). For fuller details and discussion of results the original paper should be consulted. The following outline, however, contains all the essential features:

Ten to twenty grams of soil are left in contact with 100–200 cc. of *N* NaCl for about an hour with occasional stirring. The suspension is then filtered and washed with about 500 cc. of the same solution on the filter paper. It is finally washed with a few cubic centimeter of 0.1 *N* NaCl solution, and when all of it has been drained off the remaining quantity is displaced with 10–20 cc. of water, and 0.1 *N* NaOH is gradually run in till it is alkaline to phenolphthalein (used as an external indicator, or by putting a drop of the indicator directly on the suspension). The suspension is then mechanically shaken for one hour or left for five to six hours with occasional shaking. Mechanical shaking or rubbing with a rubber pestle seems to be essential for certain red soils. For all other soils it could be dispensed with.

It is not necessary to use pure NaCl for replacement: sodium chloride containing not more than 1 per cent of calcium or magnesium salts is equally effective and can be easily prepared from ordinary common salt by adding Na_2CO_3, when the bulk of calcium and megnesium salts are precipitated. Finally the excess of Na_2CO_3 is just neutralized by adding dilute HCl. The details of the method can be varied to suit individual taste; what is required is to replace all or at least a greater part of the exchangeable ions by sodium in the clay complex.

The method of dispersion outlined was used for comparing the hydrometer and the pipette method, 2 per cent suspension being used in every case. Thirteen soils were used for the purpose. These were selected out of 48 samples with the object of having those soils which showed the greatest difference in their mechanical composition. The comparison, therefore, is even more rigid than would appear from the actual number of samples used. Two hydrometers were used, which will be referred to as No. I and No. II The bulb of the former was 9 cm. long and had a volume of 55 cc.; the latter had a bulb 6 cm. long and a volume of 74 cc. Both had a mean resting position 50 cm. below the surface.

The results given in table 1 show a very good agreement between the pipette and the hydrometer method. There is some discrepancy in the case of very coarse fractions in certain soils, but the reliability of the pipette method for particles of a limiting diameter above 0.04 mm. is questionable, for the time

TABLE 1

Mechanical analyses of soils by the hydrometer as well as the pipette method*

SOIL	METHOD	SUMMATION PERCENTAGES OF PARTICLES OF DIAMETER								
		0.002 mm.	0.004 mm.	0.006 mm.	0.008 mm.	0.01 mm.	0.02 mm.	0.04 mm.	0.06 mm.	0.08 mm.
P.C. 1	Pipette	11.3	16.0	23.6	29.0	34.6	58.1	86.3	97.6	97.4
	Hydrometer I	11.7	17.1	23.8	29.7	35.1	56.7	83.0	94.5	95.4
P.C. 2	Pipette	....	62.2	65.1	67.8	70.2	78.4	86.5	89.6	89.7
	Hydrometer I	....	62.0	67.5	70.8	73.4	81.7	86.8	88.0	89.7
P.C. 7	Pipette	....	26.6	31.4	35.3	38.6	54.2	71.4	83.6	....
	Hydrometer I	....	26.8	33.1	37.0	40.8	53.1	68.9	79.5	....
P.C. 10	Pipette	....	51.1	53.4	55.3	56.4	61.6	70.2	78.6	....
	Hydrometer I	....	48.9	54.0	55.2	56.1	62.9	68.0	71.4	....
P.C. 12	Pipette	7.2	12.5	14.6	16.4	18.0	22.9	36.7	49.9	60.7
	Hydrometer I	8.1	11.0	14.4	14.4	15.7	21.7	31.4	38.2	50.1
P.C. 19	Pipette	42.4	45.9	49.5	51.9	54.0	59.4	68.0	74.5	80.7
	Hydrometer I	40.8	44.6	46.3	48.4	51.0	57.8	65.0	68.0	71.8
P.C. 44	Pipette	....	9.7	11.7	13.9	15.7	27.6	54.2	73.8	86.2
	Hydrometer I	....	10.6	12.3	14.0	17.0	26.8	48.4	66.3	79.1
P.C. 20	Pipette	6.5	7.9	9.1	10.3	11.1	15.7	26.6	35.9	44.2
	Hydrometer I	8.1	8.5	10.2	11.9	12.3	18.3	26.3	31.4	40.8
P.C. 34	Pipette	11.3	18.8	25.0	29.6	35.0	54.2	74.6	81.4	85.0
	Hydrometer I	11.0	17.4	22.1	27.2	33.1	47.2	65.9	73.5	79.5
P.C. 39	Pipette	8.5	10.2	11.4	11.7	12.4	18.0	24.7	34.4	49.9
	Hydrometer I	....	10.1	10.7	12.5	12.5	16.9	22.5	28.7	45.0
	Hydrometer II	8.1	9.3	10.2	11.5	13.6	17.0	20.4	27.2	47.6
P.C. 27	Pipette	....	57.7	59.3	61.7	61.7	69.6	73.7	76.9	79.2
	Hydrometer I	....	55.0	58.8	61.6	63.2	69.8	73.7	76.4	78.6
	Hydrometer II	....	56.5	59.5	60.3	62.1	68.4	73.1	75.7	79.9
P.C. 45	Pipette	17.7	22.9	26.6	30.0	33.4	44.5	67.3	81.1	....
	Hydrometer I	....	20.7	25.3	30.3	33.4	46.1	64.5	78.4	87.6
	Hydrometer II	16.0	18.6	25.8	28.5	29.9	44.5	65.9	78.3	86.4
P.C. 29	Pipette	63.0	68.5	70.5	74.7	76.2	84.3	90.4	90.5	90.7
	Hydrometer I	....	64.9	71.5	74.8	77.5	85.8	91.8	92.4	92.4
	Hydrometer II	60.8	66.3	70.6	74.0	76.5	84.2	89.3	91.8	93.5

* All values refer to air-dry soil samples.

taken up in actually pipetting out a sample is large as compared to the settling velocity of the particles and it is much more likely that the hydrometer might give more reliable values in the case of such fractions. It must be admitted, however, that some disturbance in the settling particles due to the presence of the hydrometer is inevitable (4), and the low values obtained in certain cases may be partly due to that. Some error is also possible on account of the settling of the particles on the hydrometer itself tending to make it sink lower; but curiously enough the disturbance due to this cause is very little if the (NaCl-NaOH) pretreatment is used for dispersion; somehow the particles are rendered more slippery and less likely to lodge on the hydrometer. Soils merely shaken with water showed greater disturbance due to this cause.

The making and filling of the hydrometer is not difficult and a person possessing moderate skill in glass blowing can complete one in about three hours. The calibration is done by the help of a soil of known mechanical composition; a 2 per cent suspension of such a soil can be kept for this purpose.

Since the reading of the hydrometer in water must vary with the temperature, the sedimenting cylinder is kept in a water bath, through which water from the tap is kept flowing. This is quite sufficient to keep the temperature reasonably constant and no thermostat is necessary. The zero of the instrument, i.e. the reading in water, is taken just before starting the measurement by the help of a cylinder containing water and kept in the same tank. Or this can be ascertained once for all by taking readings with water at different temperatures and working out the change in the hydrometer reading per unit change in temperature.

The sedimentation cylinder can be made from a wide glass tube (about 6–7 cm. diameter) and closed at one end. The settling velocity corresponding to different fractions is adjusted at the working temperature by the help of the table given by Puri and Amin (9) or calculated from the nomographs given by Crowther (5). It is to be remembered that during sedimentation the hydrometer also moves downward for about 5–6 cm. in a 2 per cent suspension but no serious error is introduced if the mean position of the middle point is taken as the basis for computing settling times. Of course, if the summation percentages are plotted against settling velocities (v), then the position of the hydrometer can be ascertained at any time during sedimentation and v calculated therefrom.

Half a dozen hydrometers can be conveniently handled for routine work and should prove the quickest means of mechanical analysis, giving directly the summation percentage curves for soils; the results being directly comparable to the pipette method.

SUMMARY

A new type of hydrometer for the mechanical analysis of soils has been described. It consists of a short bulb (6–8 cm. long) and a long, thin stem (50–60 cm. long). The readings are taken with a special device accurate to the fraction of a millimeter.

The new hydrometer gives results closely agreeing with the standard pipette method and can replace the latter for routine mechanical analysis of soils effecting a great saving in time and labor.

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REPLACEABLE BASES IN THE SOILS OF SOUTHEASTERN MINNESOTA AND THE EFFECT OF LIME UPON THEM[1]

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This paper reports a study of the base capacity, the degree of unsaturation and the effect of different amounts of lime on it, and the relation of the degree of unsaturation to the hydrogen-ion concentration of the soils of southeastern Minnesota.

EXPERIMENTAL

Selection of methods

To determine the replaceable base the soil is either leached, or first treated with a preliminary solution and then leached, with some cation in solution until replacement ceases. Then the amounts of base removed in the leachate are determined by the usual quantitative methods. This may be, and in some cases is, checked by replacing the first base by a second and similar treatment with a different base or, in cases where ammonium chloride has been used, by the determination of the nitrogen. Gedroiz (5) first used ammonium chloride and large samples but later developed a method (6) in which he used hydrochloric acid and relatively small samples. Hissink (9) has used barium hydroxide and barium chloride to determine not only the replaceable base but also the exchange capacity. Kappen (13) has suggested hydrochloric acid to determine the base content. Lately Kelley and Brown (14), working on exchange capacity, have used barium hydroxide, ammonium chloride, and barium chloride.

Because of the conflicting evidence of different investigators in the use of the various methods it appeared desirable first to compare the results obtained by two of the methods on the same soils. The earlier methods of Gedroiz (5) were rejected as being too long and tedious since the large bulk of the leachate made accurate analysis difficult. The later method developed by him (6) was compared with a method suggested by Parker (20).

In the Parker method for exchange capacity 10 gm. of soil are treated at room temperature with 100 cc. of 0.1 *N* Ba $(OH)_2$, for a period of 16 to 18 hours. The suspension is then filtered by suction in a Gooch crucible. After all of the soil has been transferred to the crucible it is leached with 250 cc. of neutral

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N NH_4Cl. The soil and crucible are then washed with ethyl alcohol until free of chlorides, the soil is transferred to a Kjeldahl flask, and the NH_4 is determined by distillation with MgO.

For the determination of the replaceable base content, 5 or 10 gm. of soil are placed in a Gooch crucible and leached with 250 cc. of neutral N $BaCl_2$. The leachings are titrated with 0.1 N Ba $(OH)_2$ to determine the amount of hydrogen replaced by the barium, phenolphthalein being used as indicator. The soil is then leached with N NH_4Cl until the leachings give no test for barium. Barium in the NH_4Cl leaching is determined as $BaSO_4$. Expressed in chemical equivalents, the amount of barium absorbed minus the hydrogen replaced gives the replaceable base content of the original soil. The replaceable hydrogen is considered to be the difference between the exchange capacity of the soil and the content of replaceable bases in the soil.

Gedroiz has suggested the use of 0.05 N HCl as a substitute for the normal ammonium chloride method. The hydrogen ion replaces the bases from the soil with little effect upon the bases not in the soil complex. The procedure is as follows: 15–50 gm. of soil are mixed with about 50–100 cc. of 0.05 N HCl. The soil is brought on to a filter and leached until there is a complete substitution of calcium. If the soil does not contain large amounts of alkaline earth carbonates 500 to 2,000 cc. of filtrate may be expected, depending upon the percentage of zeolitic bases in the soil. Several cubic centimeters of concentrated nitric acid are added to destroy the organic matter, the solution is evaporated to dryness, and the residue is heated for an hour at 125°C. to render the silica insoluble. The residue is treated with hydrochloric acid and filtered. An aliquot is used for the determination of Ca and Mg, and another for Na and K. It must be remembered that a solution of 0.05 N HCl extracts some iron and aluminum from the soil and that these must be removed before the calcium and magnesium are determined (6).

In the calculation of the replaceable bases by this method the iron and aluminum were not included, as Gedroiz points out that the hydrolytic effects from the hydrochloric acid are too great to allow a reasonable estimation of these elements, which have at best only a doubtful claim to true replaceable form. Nor have we attempted to determine the potassium and sodium, since the preliminary work upon the samples indicated that the amount in exchangeable form in these soils is insignificant.

The total hydrogen or unsaturation was determined by means of a method proposed by Dr. Richard Bradfield, as yet unpublished, in which it is assumed that an amount of base equal to the hydrogen present in the base complex can be replaced by a buffer solution of 0.01 N NH_4OH using N NH_4Cl as the diluent. It is comparatively simple and rapid and it is possible to handle a large number of samples at one time. The method in detail is as follows: 2 gm. of soil is treated with 100 cc. of the 0.01 N NH_4OH—N NH_4Cl buffer and shaken, the suspension allowed to stand over night, and then 50 cc. siphoned off. This is titrated with 0.01 N HCl, an alcoholic solution of methyl red being used as indicator. The titration is carried to a point just below the violet red. The difference between the number of cubic centimeters of acid used and the original 50 cc. of base is equal to the milliequivalents of hydrogen present.

The hydrogen-ion concentration was determined electrometrically by means of a potentiometer using a hydrogen electrode. Air-dried samples, the moisture content of which is shown in table 2, were used in all determinations of replaceable base, replaceable hydrogen, and hydrogen ions.

Comparison of results by different methods

The data on three soils using the above methods for replaceable bases and base capacity are presented in table 1. It will be seen that very similar results were obtained by the different methods. The replaceable base as determined by the Parker and Gedroiz methods agreed closely. The latter provides a simpler and more rapid means of determining the replaceable bases and is as exact. The Bradfield method for the determination of hydrogen gave results slightly lower than the Parker method but ranks the soils in the same order. Because of their convenience and their rapidity the Gedroiz and Bradfield methods were used throughout the work in this study.

TABLE 1

Milliequivalents of replaceable bases in three soils as determined by different methods

SOIL	REPLACEABLE BASE BY PARKER METHOD	REPLACEABLE BASE BY GEDROIZ HCl METHOD	HYDROGEN BY PARKER METHOD	HYDROGEN BY BRADFIELD METHOD
19039	20	19 7	10	8 3
18908	16	17 4	16	13 0
19811	13	12 2	8	7 4

Soils used

The soils used in this investigation were taken from 28 fields in southeastern Minnesota upon which the University of Minnesota has conducted liming experiments. The fields were widely distributed and represented soil types in a triangular area 125 miles long and 90 miles wide at its widest part (fig. 1).

On five experimental fields, designated as E 1, E 2, E 3, E 4, and E 5, the liming experiments were detailed and included liming at rates varying from 1 to 8 tons an acre of finely ground limestone. On these, composite samples from the plots receiving 1, 2, 3, 4, and 8 tons of finely ground limestone as well as those from untreated plots were studied. The lime on all except E 5 had been applied in 1921, and on E 5, in 1920, a year earlier. The samples were collected in 1926, 5 years after the lime had been applied. The treatments were in triplicate and the samples used for analysis were composites taken from the three plots receiving the same treatment. They were taken in 6-inch sections from the surface to a depth of 3 feet.

On the 23 demonstration fields, designated by the letters O, D, and L, only one rate of liming, in most cases 4 tons, was used and only samples from the untreated portion of the fields were studied. The samples were composites from several borings and represented the first and second 6-inch sections and the second foot. The lime was applied in 1924 and the samples were taken 2 years later.

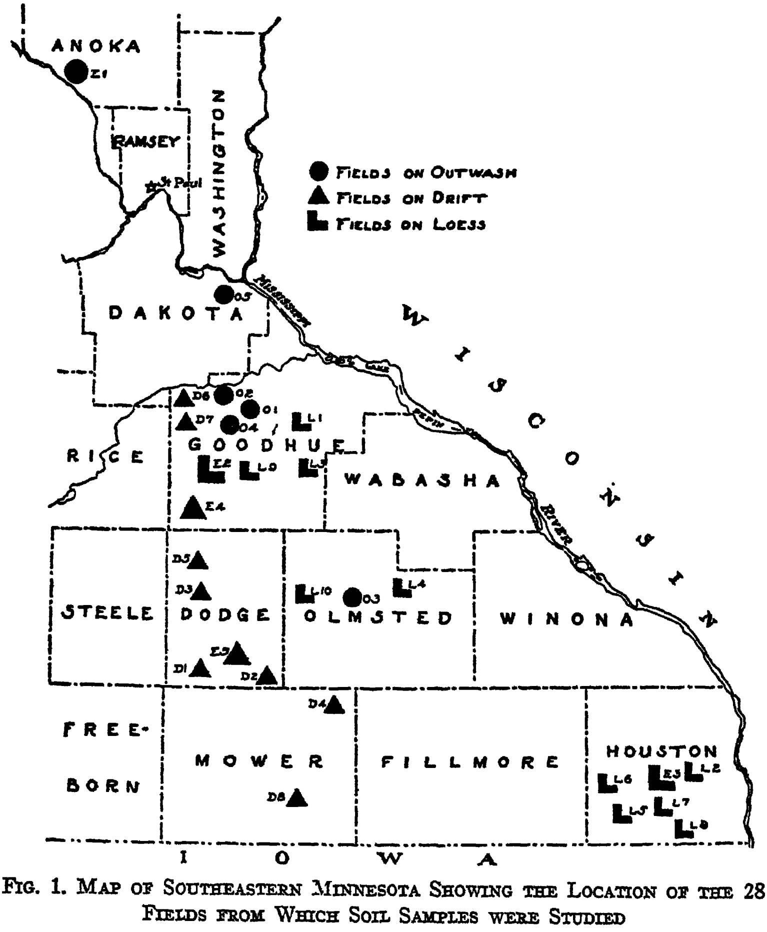

Fig. 1. Map of Southeastern Minnesota Showing the Location of the 28 Fields from Which Soil Samples were Studied

The soils of the majority of the fields had been developed upon glacial drift or upon outwash left during the recession of the Iowan and Late Wisconsin glaciers. The others had been developed upon loess.

In texture the surface soils vary from loamy sand to silty clay loam (table 2). The coarsest was the Merrimac loamy sand of field E 1, with a moisture equivalent of 3.60. The texture of the subsoils also varied widely. Within the same textural class the variations were not so great. With one exception, the texture of the 2-foot profile of each of the fields on outwash is uniform (table 2). In the case of O 4 the second foot is considerably coarser than the first foot as shown by a moisture equivalent of 11 as compared to 18 and 20 for the 0–6 and 7–12 inch layers.

TABLE 2

Texture, reaction, and moisture content of air-dry samples from three depths in the 28 fields

FIELD	TEXTURAL CLASS	MOISTURE EQUIVALENT			REACTION (pH)			MOISTURE PER CENT		
		0–6 inches	7–12 inches	13–24 inches	0–6 inches	7–12 inches	13–24 inches	0–6 inches	7–12 inches	13–24 inches
Soils developed on glacial outwash										
E 1	Loamy sand	3.6	3.9	3.0	5.7	6.3	6.4	0.53	0.55	0.50
O 1	Loamy sand	4.0	5.0	6.0	6.4	6.2	6.2	0.59	0.59	0.56
O 2	Sandy loam	13.0	18.0	15.0	5.6	6.0	6.0	1.44	1.52	1.55
O 3	Sandy loam	18.0	17.0	15.0	5.3	5.9	6.2	1.26	1.28	1.24
O 4	Sandy loam	18.0	20.0	11.0	5.6	5.8	6.0	1.55	1.6	1.12
O 5	Sandy loam	20.0	20.0	18.0	5.5	5.7	5.8	1.56	1.60	1.57
Soils developed on loess										
L 1	Silt loam	24.0	23.0	24.0	6.1	6.3	5.9	1.60	1.40	1.52
L 2	Silt loam	22.0	25.0	26.0	6.1	6.1	5.9	1.64	2.10	2.65
E 2	Silt loam	29.0	27.0	25.0	5.7	6.7	6.3	2.94	2.74	2.95
L 3	Silt loam	25.0	24.0	24.0	6.1	6.0	5.7	1.82	1.82	2.32
L 4	Silt loam	23.0	23.0	20.0	5.5	5.7	5.5	2.32	1.84	2.17
L 5	Silt loam	23.0	24.0	25.0	6.0	5.9	5.5	1.66	2.07	2.39
L 6	Silt loam	28.0	28.0	28.0	5.4	5.6	5.8	2.16	2.38	2.63
L 7	Silt loam	26.0	27.0	26.0	5.5	5.5	5.5	1.95	1.95	1.98
L 8	Silt loam	25.0	27.0	28.0	5.4	5.5	5.6	1.96	2.05	1.41
E 3	Silt loam	24.0	23.0	25.0	5.4	5.1	5.2	2.65	2.60	2.70
L 9	Silt loam	28.0	27.0	21.0	6.8	6.3	5.9	2.04	2.03	2.09
L 10	Silt loam	24.0	24.0	14.0	5.5	5.6	5.4	2.34	2.28	1.66
Soils developed on glacial drift										
D 1	Silt loam	22.0	19.0	18.0	5.6	5.7	5.6	1.99	1.06	1.85
D 2	Silty clay loam	25.0	24.0	21.0	5.5	5.3	5.2	2.48	2.22	2.17
D 3	Silty clay loam	27.0	27.0	16.0	6.0	6.0	6.1	2.06	2.06	1.75
D 4	Silty clay loam	26.0	25.0	22.0	5.4	5.5	5.6	2.58	2.18	1.97
D 5	Silty clay loam	33.0	30.0	27.0	5.4	5.6	6.6	2.94	2.77	2.75
D 6	Silty clay loam	25.0	26.0	25.0	5.9	6.1	6.2	0.69	1.63	2.25
E 4	Silty clay loam	31.0	30.0	27.0	5.3	5.8	5.8	3.40	3.34	3.03
E 5	Silty clay loam	33.0	30.0	26.0	5.4	5.3	5.5	3.40	3.40	3.16
D 7	Silty clay loam	33.0	31.0	27.0	5.6	5.8	5.9	2.52	2.50	2.28
D 8	Silty clay loam	34.0	27.0	25.0	6.8	7.0	7.5	4.13	3.53	2.75

The soils developed on glacial drift had as a rule a subsoil somewhat lighter than the surface 6 inches (table 2). With soils developed on loess the subsoil

TABLE 3

Exchange capacity and moisture equivalent of air-dry samples from the 28 fields

DEPTH	GLACIAL OUTWASH			LOESS			GLACIAL DRIFT		
	Field	Moisture equivalent	Exchange capacity	Field	Moisture equivalent	Exchange capacity	Field	Moisture equivalent	Exchange capacity
inches			*m.e.**			*m.e.*			*m.e.*
0–6	E 1	3.6	6.4	L 1	24.0	18.7	D 1	22.0	24.9
7–12		3.9	4.8		23.0	18.4		19.0	20.9
13–24		3.0	4.3		24.0	18.3		18.0	18.6
0–6	O 1	4.0	10.9	L 2	22.0	18.8	D 2	25.0	26.0
7–12		5.0	8.6		25.0	24.1		24.0	23.3
13–24		6.0	11.7		26.0	21.0		21.0	21.2
0–6	O 2	13.0	15.9	E 2	29.0	22.5	D 3	27.0	28.7
7–12		18.0	15.5		27.0	19.5		27.0	27.2
13–24		15.0	14.7		25.0	19.4		16.0	19.7
0–6	O 3	18.0	18.5	L 3	25.0	24.5	D 4	26.0	30.8
7–12		17.0	13.9		24.0	19.5		25.0	31.4
13–24		15.0	11.9		24.0	18.9		22.0	25.0
0–6	O 4	18.0	18.5	L 4	23.0	25.2	D 5	33.0	30.9
7–12		20.0	20.5		23.0	20.0		30.0	27.5
13–24		11.0	12.0		20.0	24.2		27.0	22.8
0–6	O 5	20.0	18.9	L 5	23.0	25.6	D 6	25.0	31.2
7–12		20.0	19.0		24.0	23.6		26.0	28.9
13–24		18.0	14.0		25.0	25.7		25.0	26.5
0–6		....	....	L 6	28.0	25.8	E 4	31.0	31.5
7–12		....	....		28.0	24.0		30.0	29.2
13–24		....	....		28.0	25.6		27.0	26.6
0–6		....	....	L 7	26.0	26.8	E 5	33.0	32.4
7–12		....	....		27.0	25.2		30.0	24.3
13–24		....	....		26.0	25.6		26.0	22.3
0–6		....	....	L 8	25.0	26.9	D 7	33.0	34.0
7–12		....	....		27.0	27.4		31.0	31.9
13–24		....	....		28.0	24.3		27.0	24.4
0–6		....	....	E 3	24.0	27.7	D 8	34.0	48.0
7–12		....	....		23.0	23.9		27.0	45.1
13–24		....	....		25.0	24.5		25.0	28.5
0–6		....	....	L 9	28.0	28.9		....	....
7–12		....	....		27.0	27.1		....	....
13–24		....	....		21.0	24.5		....	....

* m.e. in this and subsequent tables indicates milliequivalents per 100 gm. soil.

TABLE 3—*Concluded*

DEPTH	GLACIAL OUTWASH			LOESS			GLACIAL DRIFT		
	Field	Moisture equivalent	Exchange capacity	Field	Moisture equivalent	Exchange capacity	Field	Moisture equivalent	Exchange capacity
inches			*m.e.**			*m.e.*			*m.e.*
0–6		....	....	L 10	24.0	31.2		....	....
7–12		....	....		24.0	26.5		....	....
13–24		....	....		14.0	16.5		....	....
0–6	Average	12.7	14.8	Average	25.0	25.2	Average	29.3	31.8
7–12		13.6	13.7		25.0	23.2		27.2	29.0
13–24		11.3	11.6		23.6	22.3		23.7	23.6

was usually as heavy as the surface soil. In a few fields, such as L 2, the subsoil had a higher moisture equivalent than the surface 6 inches, whereas on another field, L 10, the subsoil was much coarser than the surface layer.

The reaction of the soils varied from acid on fields E 4 and O 3, pH 5.3, to close to neutral on D 8, pH 6.8 (table 2). In the subsoil the variation was from a pH of 5.1 on field E 3 to a pH of 7.5 on field D 8. In general the pH rose in the lower depths. The soils on glacial drift were from calcareous parent material and in some fields, such as E 4, occasional fragments of limestone were found at the 36-inch level.

Exchange capacity

The exchange capacity, which is the total exchangeable base including hydrogen in the soil, is calculated as milliequivalents of base per hundred grams of air-dry soil. In the soils used in the study the exchange capacity varied widely. The values ranged from 6 to 48 m.e. and in general the variation can be correlated rather closely with texture of the sample (table 3). The lowest capacity of 6 m.e. was found on field E 1, which is a Merrimac loamy fine sand with a moisture equivalent of 3.6, and the largest amount, 48 m.e., was in the silty clay loam with a moisture equivalent of 34 from field D 8. This wide range and its close correlation with the texture has been found generally by workers in base exchange (14, 20).

For the measure of the colloidal material present the moisture equivalent of the sample was used. Table 3 gives the base capacity and the moisture equivalents of these soils. It is evident that the relationship is close. Especially is this true in the case of the sandy loams, as for instance fields O 3 and O 4, which have moisture equivalents of 18 and base capacities of 18 m.e. For silt loams and silty clay loams the agreement is not quite so close.

The exchange capacity of the sands was between 6 and 10.9 m.e., the lower value being for field E 1 and the higher for O 1 (table 3). The sandy loams showed exchange capacities of about 18 m.e. One, field O 2, was 15.9 m.e.

The texture was somewhat coarser than that of the other fields of the textural class as indicated by a moisture equivalent of 12.

It is more difficult to set a definite capacity for the heavier soil types since the variation is wide. In the silt loams developed on loess the fields L 1 and L 2 had an exchange capacity of 18 m.e. and L 10 had one of about 31. In general the values were about 25 m.e. In the silty clay loams on the glacial drift the variations were still greater, from 26 to 48 m.e. in 100 gm. of air-dry soil (table 3). Field D 8 having 48 is rather unusual, since the base capacity found in this group of soils averaged 32 m.e., and the majority of samples from the different fields did not vary much from this figure.

The variation in exchange capacity on individual fields as shown by samples from fields E 2 and E 4 was nearly as great as that found between fields of one textural class (table 4). On E 4 the two control plots had values of 31 and 36 m.e. in the first 6 inches and of 18.7 and 26 in the 19–24 inch depths. The values found on the surface 6 inches of E 2 were 22 and 28.6 m.e.

TABLE 4

Variations in exchange capacity on two fields

FIELD E 2			FIELD E 4		
Depth	Exchange capacity		Depth	Exchange capacity	
	Plot A	Plot B		Plot A	Plot B
inches	*m.e.*	*m.e.*	*inches*	*m.e.*	*m.e.*
0–6	28.6	22.5	0–6	36.3	31.5
7–12	19.9	19.5	7–12	31.2	29.2
13–18	21.7	21.1	13–18	28.7	27.2
19–24	20.0	17.6	19–24	18.7	26.0

The exchange capacity was lowest in the soils developed on glacial outwash and highest in the silty clay loams on drift. The soils developed on loess occupied an intermediate position. It would appear that there is a definite correlation between the exchange capacity and the texture. The numerical value of the figure representing the milliequivalents of base present was almost identical with that of the figure expressing the moisture equivalent (table 5).

Bases present

The replaceable bases compose the saturated fraction of the base complex and the hydrogen present in the base complex forms the unsaturated fraction of it. In the soils of this area, hydrogen and calcium were present in far greater quantities than the magnesium. Together, they usually constituted from 86 to 90 per cent or even more of the total exchange capacity (table 6). In general the replaceable magnesium remained fairly constant regardless of the degree of saturation or texture. On field D 8 where there was only 14.6

per cent hydrogen there was 12 per cent of magnesium while on D 4 which had 46.5 per cent hydrogen there was only 13 per cent magnesium.

The replaceable calcium in these soils is always greater than the replaceable magnesium. In general the ratio for the lighter soils was approximately 1:6 although it showed a wide variation. In the silt loams and silty clay loams the ratio remained surprisingly constant, about 1:4, falling to 1:3 or even to 1:2 when the unsaturation was very high. In milliequivalents per hundred grams of soil the magnesium was about 1.5 in the sandy loams, usually from 3 to 6 in silt loams, and slightly higher in the silty clay loams on the drift. In a few cases however it was very low, such as on fields D 2, D 5, and L 2, where it was 1.6, 1.3 and 1.6 m.e. respectively.

The amount of calcium in replaceable form is governed by the degree of unsaturation. In these unsaturated soils the quantity found in the sandy loam varied from 6 to 9.6 with an average of about 8 m.e. In heavier types the range was wider but in all cases, except field D 2 surface soil and a few subsoils, the quantity of calcium was higher than that found in the best supplied sandy loams. The range in the fine textured soils was from 8.6 to 35 m.e. with an average of about 14.

TABLE 5

Average exchange capacity and moisture equivalents of soils developed on outwash, loess, and glacial drift

DEPTH	SOILS ON OUTWASH		SOILS ON LOESS		SOILS ON DRIFT	
	Moisture equivalent	Exchange capacity	Moisture equivalent	Exchange capacity	Moisture equivalent	Exchange capacity
inches						
0–6	12.7	14.8	25.0	25.2	29.3	31.8
7–12	13.6	13.7	25.0	23.2	27.3	29.0
13–24	11.3	11.6	23.6	22.3	23.7	23.6

The amounts of replaceable base were least in the soils developed on glacial outwash, slightly greater in the loess soils, and most in soils on the drift material. Loess soils were about 2 m.e. higher than the outwash soils in each case. Drift soils had a magnesium content equal to that found in the silt loams but the calcium was 4 or 5 m.e. greater and the hydrogen about 2.

Distribution of bases in the soil profile

Although the distribution of bases throughout the profile was somewhat variable, there was in general a decrease from the surface downward (table 6). In nearly all cases the largest amount was found in the surface layer. The decrease from the surface downward may be associated with the decrease in organic matter. In cases where there was any marked textural change this was indicated by an abrupt change in the amount of base present. Thus in field O 4 the second foot was very sandy and this was accompanied by a drop

TABLE 6

Exchange capacity and replaceable bases in soils from 28 fields

DEPTH	GLACIAL OUTWASH					LOESS					GLACIAL DRIFT				
	Field	Exchange capacity	H	Ca	Mg	Field	Exchange capacity	H	Ca	Mg	Field	Exchange capacity	H	Ca	Mg
inches		*m.e.*					*m.e.*					*m.e.*			
0–6	E 1	6.4	3.2	2.5	0.7	L 1	18.7	5.5	8.7	4.5	D 1	24.9	12.6	9.4	3.0
7–12		4.8	2.0	2.1	0.6		18.4	4.2	9.6	4.6		20.9	9.1	8.7	3.1
13–24		4.3	1.9	1.8	0.6		18.3	4.9	9.5	3.9		18.6	7.4	8.2	3.0
0–6	O 1	10.9	3.4	6.6	0.9	L 2	18.8	7.4	9.8	1.6	D 2	26.0	13.4	10.9	1.7
7–12		8.6	3.6	4.0	1.0		24.1	9.5	10.4	4.2		23.3	13.3	7.5	2.6
13–24		11.7	5.3	5.5	0.9		21.0	8.8	8.8	3.5		21.2	10.9	7.5	2.8
0–6	O 2	15.9	7.1	7.2	1.6	E 2	22.5	8.6	10.1	3.8	D 3	28.7	11.9	12.9	3.9
7–12		15.5	7.8	6.8	0.9		19.5	6.0	9.8	3.7		27.2	10.6	12.2	4.4
13–24		14.7	6.3	6.5	1.9		19.4	5.3	9.7	4.4		19.7	7.9	8.5	3.3
0–6	O 3	18.5	7.0	9.6	1.9	L 3	24.5	9.6	12.3	2.6	D 4	30.8	14.3	12.6	3.9
7–12		13.9	5.1	7.2	1.6		19.5	7.6	8.9	3.0		31.4	13.6	12.1	5.7
13–24		11.9	3.9	6.9	1.1		18.9	8.9	7.6	2.4		25.0	12.0	9.5	3.6
0–6	O 4	18.6	7.9	9.3	1.4	L 4	25.2	12.8	9.4	3.0	D 5	30.9	13.9	15.7	1.3
7–12		20.5	10.6	8.9	1.4		20.0	9.1	8.5	2.4		27.8	12.0	14.0	1.5
13–24		12.0	6.2	4.6	1.3		24.2	9.1	11.4	3.7		22.8	10.7	10.0	2.0
0–6	O 5	18.9	9.6	8.5	0.8	L 5	25.6	8.1	14.7	2.8	D 6	31.2	10.2	17.3	3.7
7–12		19.0	8.3	7.9	2.8		23.6	10.0	10.5	3.2		28.9	10.6	16.8	1.6
13–24		14.0	5.6	7.8	0.7		25.7	9.8	11.7	4.2		26.5	9.8	14.4	2.3
0–6		....	....	....	....	L 6	25.8	11.4	9.9	4.4	E 4	31.5	13.8	15.2	2.5
7–12		....	....	....	....		24.0	10.8	9.8	3.4		29.2	11.4	14.1	3.7
13–24		....	....	....	....		25.6	10.6	11.3	3.7		26.6	8.1	13.5	5.0
0–6		....	....	....	....	L 7	26.8	12.5	10.5	3.8	E 5	32.4	12.0	15.8	4.6
7–12		....	....	....	....		25.2	11.8	9.6	3.8		24.3	9.8	10.7	3.8
13–24		....	....	....	....		25.6	12.6	9.2	3.8		22.3	8.3	10.5	3.5
0–6		....	....	....	....	L 8	26.9	12.9	9.9	4.1	D 7	24.0	12.8	16.8	4.5
7–12		....	....	....	....		27.4	11.2	11.5	4.7		31.9	13.0	14.7	4.2
13–24		....	....	....	....		24.3	11.5	10.7	2.1		24.4	9.6	10.6	4.1
0–6		....	....	....	....	E 3	27.7	13.0	10.6	4.1	D 8	48.0	7.0	35.2	5.9
7–12		....	....	....	....		23.9	12.4	8.5	4.1		45.1	5.5	24.0	5.6
13–24		....	....	....	....		24.5	11.8	8.3	4.4		28.5	2.5	21.5	4.5
0–6		....	....	....	....	L 9	28.9	6.3	18.1	4.5		....	....	....	...
7–12		....	....	....	....		27.1	8.5	14.9	3.8		....	....	....	...
13–24		....	....	....	....		24.5	7.0	12.6	4.9		....	....	....	...

TABLE 6—*Concluded*

DEPTH	GLACIAL OUTWASH					LOESS					GLACIAL DRIFT				
	Field	Exchange capacity	H	Ca	Mg	Field	Exchange capacity	H	Ca	Mg	Field	Exchange capacity	H	Ca	Mg
inches		*m.e.*					*m.e.*					*m.e.*			
0–6		....	....	....	....	L 10	31.2	12.6	14.9	3.7		....	....	....	...
7–12		....	....	....	....		26.5	10.1	13.2	3.2		....	....	....	...
13–24		....	....	....	....		16.5	5.6	7.9	3.0		....	....	....	...
0–6	Average	....	7.9	8.6	1.4	Average	....	10.1	11.6	3.6	Average	....	12.2	16.2	3.5
7–12		....	7.9	7.7	1.7		....	9.3	10.4	3.7		....	10.9	14.5	3.6
13–24		....	5.5	6.4	1.2		....	8.8	9.9	3.9		....	8.7	11.4	3.4

from 20.5 m.e. in the second 6 inches to 12.0 in the second foot. Likewise on field L 10 there was a change in texture at the end of the first foot as indicated by a lowering of the moisture equivalent from 24 to 14 and a drop in the amount of base from 26.5 to 16.5 m.e. In only one field was the surface 6 inches much lower in base content than the second 6 inches and that was in field L 2 where the surface was 18.8 and the second 6 inches 24.1 m.e.

Though the decrease downward was general there was usually less drop in base content in the lower depths of silt loams developed on loess than in those soils developed on glacial outwash and drift (table 6). In all profiles the average shows but a slight decrease in the second 6 inches and on the glacial drift soils a very marked drop in the second foot.

Degree of unsaturation

Hydrogen is the most powerful of all bases as a replacing cation and once it has entered the base complex only relatively great changes in the concentration of the soil solution will change the equilibrium in respect to it. In this form hydrogen comprises one of the more important types of soil acidity. Gedroiz (6) believes that much acidity is due to the hydrogen from base complex being released to react with aluminum and iron compounds. Kappen (12) on the other hand divides acidity into four groups: hydrolytic, actual acidity due to humus, and exchangeable acidity of the base complex. Nevertheless, just how much the acidity of a soil as expressed by the hydrogen-ion concentration is affected by this type of hydrogen is doubtful. Pierre (23) has shown that with certain acid-sensitive crops the growth is more closely correlated with the unsaturation than with the acidity of the soil indicated by the pH values.

The unsaturation, expressed as percentage of hydrogen in the total base, in samples used in this study is high. For the surface 6 inches the average was 40.3 per cent and equally great for the second 6 inches and second foot. It ranged from a maximum of 57 per cent on field D 2 to a minimum of 8.6

TABLE 7

Percentage unsaturation and reaction of soils from 28 fields

DEPTH	GLACIAL OUTWASH			LOESS			GLACIAL DRIFT		
	Field	Unsat-uration	pH	Field	Unsat-uration	pH	Field	Unsat-uration	pH
inches		*per cent H*			*per cent H*			*per cent H*	
0–6	E 1	50.9	5.7	L 1	29.5	6.1	D 1	50.5	5.6
7–12		42.1	6.3		22.9	6.3		43.5	5.7
13–24		35.0	6.4		26.9	5.9		39.7	5.6
0–6	O 1	31.2	6.4	L 2	39.4	6.1	D 2	51.5	5.5
7–12		41.7	6.2		39.5	6.1		57.0	5.3
13–24		45.3	6.2		41.8	5.9		51.5	5.2
0–6	O 2	44.6	5.6	E 2	38.3	5.7	D 3	41.6	6.0
7–12		50.4	6.0		30.2	6.7		39.0	6.0
13–24		42.9	6.0		24.8	6.3		40.1	6.1
0–6	O 3	37.9	5.3	L 3	39.1	6.1	D 4	46.5	5.4
7–12		36.7	5.9		39.2	6.0		43.4	5.5
13–24		32.8	6.2		47.0	5.7		47.9	5.6
0–6	O 4	42.5	5.6	L 4	50.9	5.5	D 5	45.0	5.4
7–12		50.0	5.8		45.5	5.7		43.6	5.6
13–24		51.4	6.0		37.7	5.5		47.0	6.0
0–6	O 5	51.7	5.5	L 5	31.7	6.0	D 6	32.7	5.9
7–12		43.7	5.7		42.3	5.9		36.6	6.1
13–24		39.8	5.8		38.2	5.5		37.1	6.2
0–6		....	...	L 6	44.3	5.4	E 4	43.8	5.3
7–12		....	...		45.0	5.6		39.1	5.8
13–24		....	...		41.4	5.8		30.3	5.8
0–6		....	...	L 7	46.6	5.5	E 5	37.0	5.4
7–12		....	...		46.9	5.5		40.2	5.3
13–24		....	...		49.3	5.5		36.8	5.5
0–6		....	...	L 8	48.0	5.4	D 7	37.6	5.6
7–12		....	...		40.9	5.5		40.8	5.8
13–24		....	...		47.3	5.6		39.5	5.9
0–6		....	...	E 3	46.8	5.4	D 8	14.6	6.8
7–12		....	...		49.5	5.1		12.2	7.0
13–24		....	...		45.8	5.2		8.6	7.5
0–6		....	...	L 9	21.8	6.8		....	...
7–12		....	...		31.3	6.3		....	...
13–24		....	...		28.6	5.9		....	...

TABLE 7—*Concluded*

DEPTH	GLACIAL OUTWASH			LOESS			GLACIAL DRIFT		
	Field	Unsaturation	pH	Field	Unsaturation	pH	Field	Unsaturation	pH
inches		*per cent* H			*per cent* H			*per cent* H	
0–6		....	...	L 10	40.4	5.5		....	...
7–12		....	...		37.9	5.6		....	...
13–24		....	...		34.1	5.4		....	...
0–6	Average	43.1		Average	39.7	...	Average	40.1	...
7–12		44.1			39.3	...		39.6	...
13–24		41.2			38.6	...		37.9	...

per cent on D 8 (table 7). The variation seems to be independent of the texture, as the average unsaturation is approximately equal on all textural groups.

The unsaturation in the upper 2 feet of the profile is exceedingly variable and the variation could not be correlated with either the texture or the organic matter. In those fields for which determinations were made on the third foot the percentage unsaturation in the last foot is regularly less than that in the upper 2 feet (table 8). On the heavier soils and especially on field E 4, in which the calcerous subsoil is encountered shortly below the 36-inch depth, is the decrease very marked. On the loamy sand of field E 1 this drop is less, as though excessive leaching had caused the removal of saturating cations to greater depths in the coarser soil.

Relation of unsaturation to pH

The pH as a measure of the active acidity represents the hydrogen-ion concentration in the soil solution. One would expect from the nature of the exchange complex a certain interrelation between the pH, which represents the hydrogen in the soil solution, and the degree of unsaturation, which represents the hydrogen in exchangeable form. The hydrogen in the latter case is probably potential hydrogen and as such is a source for the active hydrogen removed from the soil. There are, however, so many factors which influence the pH of a soil besides the hydrogen of the exchange complex that a close relationship is not always discernible in a consideration of only the pH and the degree of unsaturation.

In the soils used in this investigation a general relationship is found between the pH and the degree of unsaturation. That is, usually with a greater degree of unsaturation there is a corresponding lower pH. That this does not always hold may be seen from table 9. As has been mentioned, texture appears to have no effect upon the degree of unsaturation or the pH or upon the correlation of these.

Between the pH values 5.8 and 6.2 there are greater variations than either above or below this range. It is quite possible that this increased variation

TABLE 8

Percentage unsaturation on experimental fields

DEPTH	FIELD E 1	FIELD E 2	FIELD E 3	FIELD E 4	FIELD E 5
inches					
0–6	50.9	38.3	46.8	43.8	37.0
7–12	42.1	30.2	49.5	39.1	40.2
13–18	27.7	33.2	50.6	30.2	41.1
19–24	42.3	19.4	40.9	30.4	32.4
25–30	47.7	24.2	39.7	30.8	30.8
31–36	42.9	26.1	37.1	19.5	32.6

TABLE 9

Percentage unsaturation and reaction in first and second 6 inches and second foot, arranged according to increasing pH

0–6 INCHES			7–12 INCHES			13–24 INCHES		
Field	pH	Unsaturation	Field	pH	Unsaturation	Field	pH	Unsaturation
		per cent H			*per cent H*			*per cent H*
E 4	5.3	43.8	E 3	5.1	49.5	E 3	5.2	46.0
O 3	5.3	37.9	E 5	5.3	40.2	D 2	5.2	51.5
E 5	5.4	37.0	D 2	5.3	57.0	L 10	5.4	34.1
E 3	5.4	46.8	D 4	5.5	43.4	E 5	5.5	37.0
L 8	5.4	48.0	L 7	5.5	46.9	L 4	5.5	37.7
L 6	5.4	49.3	L 8	5.5	40.9	L 7	5.5	49.3
D 4	5.4	46.5	L 6	5.6	45.0	L 5	5.5	38.2
D 5	5.4	45.0	L 10	5.6	37.9	D 1	5.6	39.7
D 2	5.5	51.5	D 5	5.6	43.6	D 4	5.6	47.9
O 5	5.5	51.7	D 1	5.7	43.5	L 8	5.6	47.3
L 4	5.5	50.9	L 4	5.7	45.5	L 3	5.7	47.0
L 7	5.5	46.6	O 5	5.7	43.7	E 4	5.8	30.3
L 10	5.5	40.4	E 4	5.8	39.1	L 6	5.8	41.4
O 2	5.6	44.6	O 4	5.8	50.0	O 5	5.8	39.8
O 4	5.6	42.5	D 7	5.8	40.8	D 7	5.9	39.5
D 7	5.6	37.6	O 3	5.9	36.7	L 2	5.9	41.8
D 1	5.6	50.5	L 5	5.9	42.3	L 1	5.9	26.9
E 2	5.7	38.3	L 3	6.0	39.2	L 9	5.9	28.6
E 1	5.7	50.9	O 2	6.0	50.4	O 2	6.0	42.9
D 6	5.9	32.7	D 3	6.0	39.0	O 4	6.0	51.4
L 5	6.0	31.7	D 6	6.1	36.6	D 5	6.0	47.0
D 3	6.0	41.6	L 2	6.1	39.5	D 3	6.1	40.1
L 3	6.1	39.1	O 1	6.2	41.7	O 3	6.2	32.8
L 2	6.1	39.4	L 9	6.3	31.3	O 1	6.2	45.3
L 1	6.1	29.5	L 1	6.3	22.9	D 6	6.2	37.1
O 1	6.4	31.2	E 1	6.3	42.1	E 2	6.3	26.0
D 8	6.8	14.6	E 2	6.7	30.2	E 1	6.4	36.0
L 9	6.8	21.8	D 8	7.0	12.2	D 8	6.5	8.6

may be due to a considerable extent to the fact that this is the borderline between distinctly acid soils and those which are neutral. In this range the degree of unsaturation goes to as high as 51.4 per cent, a value equal to any except that of field D 2, and as low as 29.6 per cent, which is no greater than that found in one sample from field E 2 with a pH of 6.7.

Near the neutral point the unsaturation was rather uniformly low, usually below 30 per cent, a limit which has been generally found by other workers. This would seem to indicate that the basic reacting substances in the soil are able to neutralize the acidity of the soil as measured by the hydrogen-ion concentration before they completely saturate the base complex. That is to say that even though the pH is 7.0 or above there may still be hydrogen present in exchangeable form which is difficultly replaceable and is not replaced before a pH of approximately 8.5 is reached (13).

In the more acid soils the degree of unsaturation was almost invariably high. In all cases it was greater than 30 per cent and frequently as high as 50 per cent. With five exceptions, L 1, L 9, L 10, D 6, and E 4, all of the samples which had a pH below 6.0 had an unsaturation above 35 per cent. Of those below a pH of 5.5 the great majority were above 44 per cent unsaturated.

Harada (7) has reported a close agreement between the pH and the degree of unsaturation. Other investigators (11), however, have observed the same general relationship reported in the foregoing. Wilson (30) working with soils of New York found a correlation between the exchangeable calcium and the hydrogen-ion concentration. With the soils used in this study the relationship between exchangeable lime and pH is less marked than between the pH and the degree of unsaturation. Our data would indicate that the bases in the exchange complex in a soil do not change as rapidly as the pH and that soils of the same pH value may have widely different degrees of unsaturation.

Effect of lime on the exchange complex

A comparison of the soils from the limed plots with those from the untreated plots on the same field brings forth some interesting data concerning the effect of lime. The samples for this study were taken on the five experimental fields from plots receiving 1, 2, 3, 4, and 8 tons an acre of finely ground limestone as well as from untreated or control plots. The lime had been applied in 1920 or 1921, alfalfa seeded in the spring of 1922, and the fields kept continuously in alfalfa until after the samples were taken in the autumn of 1926. On fields E 1, E 2, E 3, and E 4 finely ground limestone, all of which passed through a 100-mesh sieve, was applied. Hydrated lime was applied on field E 5. The composition of the liming material is presented in table 10. With all the fields except E 5 two sets of untreated plots have been used as controls throughout the study.

Rost and Fieger (25) calculated that the hydrated lime was equal in effectiveness to twice the amount of finely ground limestone. When the CaO equivalent for the MgO is calculated the content of the CaO for the two forms is: finely ground limestone, 41.95 per cent, and hydrated lime, 88.30 per cent.

The soils of these fields varied in texture from a loamy sand on field E 1 to a silty clay loam on field E 5 (table 11). The reaction of the surface soil of the fields as expressed by the hydrogen-ion concentration was acid and ranged from pH 5.7 to 5.3 (table 11). On fields E 1, E 2, and E 4 it decreased below the surface 6 inches and on field E 4 below the 18-inch level. In field E 3 the reaction was much the same throughout the profile. In organic matter the silty clay loams on E 4 and E 5 were best supplied. Fields E 2 and E 3 carried somewhat lower amounts and E 1 the least, having only 1.0 per cent in the surface section (table 12).

TABLE 10

Chemical composition of liming materials used

MATERIAL	INSOLUBLE IN HCl	CaO	MgO
	per cent	*per cent*	*per cent*
Finely ground limestone	22.21	30.14	8.71
Hydrated lime	0.06	47.11	29.66

TABLE 11

Moisture equivalent and reaction of samples from untreated plots of the experimental fields

DEPTH	FIELD E 1		FIELD E 2		FIELD E 3		FIELD E 4		FIELD E 5	
	Moisture equivalent	pH	Moisture equivalent	pH	Moisture equivalent	pH	Moisture equivalent	pH	Moisture equivalent	pH
inches										
0–6	3.6	5.7	29.2	5.7	23.6	5.4	30.7	5.3	32.8	5.4
7–12	3.9	6.3	26.9	6.7	22.5	5.2	29.6	5.8	29.5	5.3
13–18	3.0	6.4	25.1	6.3	24.7	5.1	26.9	5.7	26.1	5.4
19–24	3.0	6.1	25.0	6.4	24.8	5.5	26.8	6.1	26.0	5.8
25–30	2.7	6.4	23.9	6.4	25.6	...	22.2	7.0	20.1	5.8
31–36	2.7	7.3	23.6	6.9	25.5	5.3	22.0	6.6	20.0	6.1

TABLE 12

Percentage of organic matter (N × 20) in representative profiles of the experimental fields

DEPTH	FIELD E 1	FIELD E 2	FIELD E 3	FIELD E 4	FIELD E 5
inches					
0–6	1.0	5.4	3.8	6.2	7.6
7–12	0.6	4.0	2.0	4.4	4.8
13–24	0.2	2.2	1.0	2.0	2.2
25–36	0.2	1.0	0.6	1.0	0.8

The exchange capacity of the untreated plots varied rather widely, as is

TABLE 13

Exchange capacity in milliequivalents per 100 gm. of air-dry soil from plots on experimental fields

DEPTH	LIME 8 TONS	LIME 4 TONS	LIME 3 TONS	LIME 2 TONS	LIME 1 TON	Untreated	
inches						Set A	Set B
				Field E 1			
0–6	11.25	8.77	5.94	8.57	7.65	6.41	7.85
7–12	7.78	6.13	7.29	5.27	4.71	4.77	6.64
13–18	6.10	7.86	6.63	4.67	4.93	4.52	5.30
19–24	5.69	6.10	3.85	6.47	3.90	4.06	5.19
25–30	6.35	.....	.....	3.98	.....	.....	5.03
31–36	4.14	.....	.....	5.57	.....	....	6.52
				Field E 2			
0–6	34 5	30.1	29.2	31.9	26.0	28 6	22.5
7–12	15 6	24.6	25.5	23.2	24.9	19.9	19.5
13–18	20 8	17.5	24.2	19.1	22.4	21.7	21.1
19–24	16.1	23.0	23.1	24.0	22.9	20 0	17.6
25–30	13 6	26.4	26.6	24.7	21.0	24.7	16.6
31–36	17.5	17.7	23.5	19.7	22.9	18.8	20.7
				Field E 3			
0–6	36 86	27.22	25.71	25.78	27.63	27.42	27.74
7–12	22.86	25.44	24.68	20 84	22.75	23.87	25.04
13–18	24.20	22.79	22.67	20.46	20.51	23.57	27.07
19–24	24.30	21.72	22.11	21.13	20.92	24.31	22.00
25–30	25.81	23.81	24.30	22.52	24.59	23 91	22.39
31–36	22.69	24.27	30.37	22.79	27.17	25.49	22.09
				Field E 4			
0–6	33.9	34.8	32.9	33.9	33.0	36.3	31.5
7–12	28.0	32.0	32.5	28.5	28.0	31.2	29.2
13–18	26.2	27.3	29.4	27.0	25.6	28.7	27.2
19–24	20.9	24.6	22.4	27.2	26.7	18.7	26.0
25–30	19.6	22.4	20.4	21.9	23.4	17.2	21.8
31–36	19.6	21.8	22.0	20.1	22.2	17.0	18.5
				Field E 5			
0–6	35.29	31.7	.....	33.48	.....	32.43	.....
7–12	24.23	25.30	.....	22.75	.....	24.33	.....
13–18	26.05	26.47	.....	24.87	.....	24.32	.....
19–24	20.53	20.99	.....	20.4	.....	20.33	.....
25–30	15.72	15.80	.....	18.88	.....	19.42	.....
31–36	17.41	20.63	.....	17.77	.....	19.58	.....

shown in table 13. The loamy sand of field E 1 had a base capacity of 6.8 m.e., and the silty clay loams of E 4 and E 5, both developed on glacial drift, of 31 and 36 m.e. on two sets of plots on the former and of 32 m.e. on the latter. Field E 3, a silt loam, had an exchange capacity of 27 m.e., whereas for E 2, also a silt loam, it was 28 on one set of control plots and 22 on the other. The soil of the last two fields have been developed on loess.

The 8-ton application of lime produced on all fields but E 4 an increase in the total base capacity of the soil (table 13). On that field the increase of 2 m.e. over the lower control is still less than the exchange capacity of 36.3 on the higher control. The increase in exchange capacity caused by 8 tons of lime on field E 2 was 6 m.e. over the higher control plot and of 12 over the lower one. On E 3 an increase of 9 m.e. is shown. Upon both E 1 and E 5 a rise of about 3 or 4 m.e. can be observed, although relatively this amount represents a considerably greater change on E 1 than it does on E 5, which has a greater initial capacity. Only upon field E 2 did any increase in exchange capacity appear to be caused by an application of less than 8 tons of lime. Here the 4-, 3-, and 2-ton applications gave exchange capacities greater than that of the low control sample though not much greater than that of the high one. These data confirm the findings of other investigators (26) that the addition of basic substances tends to increase the exchange capacity of the soil.

Effect of lime on replaceable calcium and magnesium

The calcium and magnesium content on the more heavily limed plots was in most cases several millequivalents higher than that in the untreated soils (tables 14 and 15). With the 8-ton application the calcium and magnesium content was 5 m.e. more than on the untreated plot on field E 1, 14 more on E 2, 11 more on E 3, and 9 more on E 5. On E 4 there was no apparent increase in the calcium and magnesium on the plots to which 8 tons of lime had been applied over that in the higher control plot. When calculated. as per cent of total base, however, the data show an increase of 6 per cent over the higher check and of 10 per cent over the lower one. On the other fields the increase ranges in percentage from 50 on field E 1 to 20 or 30 on E 2, E 5, and E 3. The amount of increase seems to be more or less governed by the base capacity of the soil, the soils with lower exchange capacities becoming more nearly saturated with calcium and magnesium upon liming than do the finer textured soils with high exchange capacities.

In general the increase of calcium and magnesium in the base complex was correlated with the amount of lime applied. Thus the 8-ton application usually produced at least twice as much increase in the calcium and magnesium as did the 4-ton treatment. On field E 4, however, the 8-, 4-, and 3-ton applications produced about equal effects.

The point at which an effect from applications of lime can be observed appears dependent upon the individual soil. Usually in the soils which were

TABLE 14

Replaceable calcium in milliequivalents per 100 gm. of air-dry soils from plots on the experimental fields

DEPTH	LIME 8 TONS	LIME 4 TONS	LIME 3 TONS	LIME 2 TONS	LIME 1 TON	Untreated	
inches						Set A	Set B
Field E 1							
0–6	8.20	4.74	3.57	5.28	3.89	2.49	3.00
7–12	3.70	3.24	3.89	2.67	2.28	2.14	3.10
13–18	2 70	3.46	3.03	2.21	2.25	1.89	2.60
19–24	3.30	2.53	1.85	2.49	2.64	1.78	2.20
25–30	2.60	.....	.....	1 78	.....	.....	2.40
31–36	2.20	.....	.....	2.85	.....	.....	2.60
Field E 2							
0–6	24.0	17.0	12.8	16.7	15.2	14.9	10.1
7–12	5.3	12.0	11.5	10.2	11.4	10.7	9.8
13–18	9.3	9.9	10.5	8.7	11.0	10.5	9.4
19–24	4.3	11.3	10.7	10.7	12.1	10.9	10.1
25–30	5.1	12.2	12.8	10.8	12.3	12.1	8.4
31–36	10.8	6.2	13.1	13.2	12.2	12.3	10.2
Field E 3							
0–6	17.31	11.70	10.63	10.03	10.67	12.00	10.60
7–12	8.74	9.17	9.00	7.88	8.67	9.96	8.50
13–18	9.30	8.38	8.21	7.78	7.17	7.49	9.20
19–24	8.90	9.03	9.24	8.42	8.56	8.42	8.30
25–30	9.70	10.31	11.24	10.50	10.60	9.74	9.60
31–36	9.20	11.49	12.53	11.60	11.67	11.38	10.42
Field E 4							
0–6	18.9	19.7	19.8	16.1	16.1	16.3	15.2
7–12	14.8	13.8	14.0	12.2	11.7	13.6	14.1
13–18	11.0	12.7	12.6	12.0	12.2	12.6	12.6
19–24	10.9	12.5	12.9	13.9	13.9	12.1	14.5
25–30	10.7	13.9	11.8	11.8	12.0	9.6	13.0
31–36	10.7	13.5	11.6	11.9	11.3	10.6	12.2
Field E 5							
0–6	22.00	17.63	.....	19.70	.....	15.80	.....
7–12	10.20	11.31	.....	9.38	.....	10.70	.....
13–18	11.10	11.42	.....	11.78	.....	10.70	.....
19–24	9.30	11.74	.....	10.67	.....	10.40	.....
25–30	8.10	9.06	.....	10.63	.....	9.90	.....
31–36	9.20	14.45	.....	10.99	.....	9.80	.....

TABLE 15

Replaceable magnesium in milliequivalents per 100 gm. of air-dry soil from plots on experimental fields

DEPTH	LIME 8 TONS	LIME 4 TONS	LIME 3 TONS	LIME 2 TONS	LIME 1 TON	UNTREATED	
inches						Set A	Set B
			Field E 1				
0–6	3.05	0.83	0.97	2.29	1.96	0.72	0.85
7–12	1.28	1.69	0.90	1.00	0.63	0.63	0.74
13–18	0.70	1.80	0.86	0.55	0.88	0.63	0.70
19–24	0.79	1.57	1.00	0.98	0.56	0.68	0.79
25–30	0.45	....	....	1.00	....	....	0.63
31–36	0.74	....	....	0.72	....	....	1.12
			Field E 2				
0–6	7.5	6.7	3.6	6.2	2.0	2.3	3.8
7–12	3.5	6.0	4.0	4.0	4.5	1.8	3.7
13–18	4.5	2.4	4.9	3.6	3.6	3.2	4.7
19–24	3.6	5.5	5.4	6.5	3.2	2.1	4.1
25–30	5.5	7.4	6.6	5.9	4.5	5.6	4.2
31–36	3.5	7.7	6.2	2.6	6.5	1.9	5.1
			Field E 3				
0–6	10.5	5.32	4.88	4.35	4.96	4.22	4.14
7–12	3.92	4.27	3.68	2.96	2.88	3.91	4.14
13–18	4.30	3.61	3.26	3.48	3.24	2.88	4.12
19–24	5.40	4.09	5.07	3.71	4.16	5.09	4.70
25–30	5.91	5.70	5.86	4.62	5.39	5.57	3.89
31–36	7.09	5.78	10.64	5.19	7.90	6.71	3.47
			Field E 4				
0–6	3.8	3.9	3.1	5.6	5.9	5.8	2.5
7–12	2.0	5.2	6.5	5.1	5.5	4.6	3.7
13–18	4.2	4.0	5.6	5.8	5.4	4.5	6.4
19–24	3.2	4.7	2.9	7.5	6.8	3.2	3.6
25–30	5.1	4.3	3.8	6.5	7.0	5.0	2.2
31–36	3.4	7.2	5.6	6.4	5.9	4.0	2.7
			Field E 5				
0–6	7.09	4.67	....	5.18	....	4.63	....
7–12	3.43	3.79	....	4.17	....	3.83	....
13–18	4.95	4.85	....	4.09	....	3.62	....
19–24	3.63	4.35	....	3.93	....	3.33	....
25–30	3.22	3.34	....	3.95	....	3.52	....
31–36	4.01	5.48	....	3.98	....	3.38	....

used in this investigation this point appeared between the 3- and 4-ton applications. Except with field E 1 it was impossible to note any change in the exchange complex, until at least 3 tons of limestone had been applied.

Effect of lime upon unsaturation and replaceable hydrogen

As was the case with calcium and magnesium entering the base complex, no effect of the lime applied was shown by a decrease in the hydrogen content and a subsequent decrease in the unsaturation until at least 3 or 4 tons of lime had been applied. Similarly also the hydrogen decrease was more or less proportional to the amount of lime applied (tables 16 and 17).

The increase in calcium and magnesium was in most, but not all cases, coincident with a decrease in hydrogen. For instance on field E 3 the replaceable hydrogen in the first 6 inches decreased 2 m.e. although the calcium and magnesium content was scarcely affected by the 3- and 4-ton applications of limestone (tables 14, 15, and 16). On field E 1 the calcium increased about 1.5 m.e. but the hydrogen did not vary. In general the increase in basic ions was accompanied by a decrease in hydrogen. The exchange was rarely equivalent. In the majority of cases the calcium and magnesium absorbed was greater than the hydrogen replaced. This discrepancy in the exchange would substantiate the conclusion mentioned before, that basic substances increase the adsorptive capacity of a soil. According to Kelley and Brown (15) the exchange of cations should be stochiometric or approximately so.

On field E 1, 1 ton of lime decreased the unsaturation as much as 4 tons of lime, although not to an equal depth. In the case of the 1-ton application the unsaturation in the second 6 inches amounts to 38.2 per cent and with the 4-ton application for the same section it is 30.8 per cent. As the unsaturation is 41 and 42 per cent in this section of the control plots one can consider the decrease with the 4-ton application as being due to the lime and not within the limits of experimental error. Neither the 1-, 2-, nor 3-ton applications of lime had any effect below the first foot on field E 1. The 4-ton application shows a quite definite effect on the second foot (table 17).

The only plots upon which the addition of lime completely saturated the base complex were those on field E 1 which received 8 tons of lime. Here in the first 6 inches there was no replaceable hydrogen or unsaturation. Below this section, however, it is difficult to indicate the point to which the lime was effective, but certainly it was effective to the end of the first foot. The pH of the plot receiving 8 tons was 8.3, which agrees well with the work of Kappen (13), who has suggested that complete saturation does not take place below a pH of 8.5. Parker (21) has taken a pH of 7.0 as the point at which complete saturation occurs.

On the other experimental fields the unsaturation in most cases was lowered to less than 30 per cent by an application of 3 or more tons of limestone. There appears to have been no effect from lime upon the unsaturation in the base complex below the surface 6-inch layer of these fields.

TABLE 16

Replaceable hydrogen in milliequivalents per 100 gm. of air-dry soil from plots on experimental fields

DEPTH	LIME 8 TONS	LIME 4 TONS	LIME 3 TONS	LIME 2 TONS	LIME 1 TON	UNTREATED	
inches						Set A	Set B
Field E 1							
0–6	0.0	3.2	1.4	1.0	1.8	3.2	4.0
7–12	2.8	1.2	2.5	1.6	1.8	2.0	2.8
13–18	2.7	2.6	2.8	2.0	1.8	2.0	2.0
19–24	1.6	2.0	1.0	3.0	0.7	1.6	2.2
25–30	3.3	....	....	1.2	....	....	2.0
31–36	1.2	....	....	2.0	....	....	2.8
Field E 2							
0–6	3.0	6.4	8.0	9.0	8.8	11.4	8.6
7–12	6.8	6.6	10.0	9.0	9.0	7.4	6.0
13–18	7.0	5.2	8.8	6.8	7.8	8.0	7.0
19–24	8.2	6.2	7.0	6.8	7.6	7.0	3.4
25–30	3.0	6.8	7.2	8.0	4.2	7.0	4.0
31–36	3.2	3.8	4.2	3.9	4.2	4.6	5.4
Field E 3							
0–6	9.4	10.2	10.2	11.4	12.0	11.2	13.0
7–12	10.2	12.0	12.0	10.0	11.2	10.0	12.4
13–18	10.6	10.8	11.2	9.2	10.1	13.2	13.7
19–24	10.0	8.6	7.8	9.0	8.2	10.8	9.0
25–30	10.2	7.8	7.2	7.4	8.6	8.6	8.9
31–36	6.4	7.0	7.2	6.0	7.6	7.4	8.2
Field E 4							
0–6	11.2	11.2	10.0	12.2	11.0	14.2	13.8
7–12	11.2	13.0	12.0	11.2	10.8	13.0	11.4
13–18	11.0	10.6	11.2	9.2	8.0	11.6	8.2
19–24	6.8	7.4	6.6	5.8	6.0	3.4	7.9
25–30	3.8	4.2	4.8	3.6	4.4	2.6	6.6
31–36	5.5	1.1	4.8	1.8	5.0	2.4	3.6
Field E 5							
0–6	6.2	9.4	....	8.6	....	12.0	....
7–12	10.6	10.2	....	9.2	....	9.8	....
13–18	10.0	10.2	....	9.0	....	10.0	....
19–24	7.6	4.9	....	5.8	....	6.6	....
25–30	4.4	3.4	....	4.3	...	6.0	....
31–36	4.2	0.7	....	2.8	....	6.4	....

TABLE 17

Effect of different amounts of lime on unsaturation of soils of the experimental fields

DEPTH	LIME 8 TONS	LIME 4 TONS	LIME 3 TONS	LIME 2 TONS	LIME 1 TON	UNTREATED	
inches						Set A	Set B
Field E 1							
0–6	0.0	36.4	23.5	11.4	23.5	49.9	50.9
7–12	35.9	19.5	34.2	30.8	38.2	41.9	42.1
13–18	44.2	33.0	41.8	42.8	36.5	44.2	27.7
19–24	28.1	32.7	25.9	46.3	17.9	39.4	42.3
25–30	51.9	....	....	30.1	....	....	47.7
31–36	28.9	....	....	35.9	....	....	42.9
Field E 2							
0–6	8.7	21.3	27.4	28.3	33.9	39.9	38.3
7–12	43.6	26.9	39.3	38.8	36.2	37.2	30.2
13–18	33.7	29.8	36.4	35.6	34.1	36.9	33.2
19–24	51.0	27.0	30.4	28.4	33.2	35.0	19.4
25–30	22.1	25.8	27.1	32.4	20.0	28.4	24.2
31–36	18.3	21.5	17.9	19.8	18.4	24.5	26.1
Field E 3							
0–6	25.3	37.4	39.6	44.2	43.4	40.8	46.8
7–12	44.6	47.1	48.6	48.8	49.2	41.8	49.5
13–18	43.8	47.3	49.4	44.9	49.2	56.0	50.6
19–24	41.1	39.5	35.2	42.5	39.1	44.4	40.9
25–30	39.5	32.7	29.6	32.8	34.9	35.9	39.7
31–36	28.2	28.8	23.7	26.3	27.9	29.0	37.1
Field E 4							
0–6	33.1	32.2	30.6	36.0	33.4	39.2	43.8
7–12	40.0	40.7	27.0	39.3	38.6	41.7	39.1
13–18	42.0	38.9	38.1	34.1	31.3	40.5	30.2
19–24	32.6	30.1	29.5	21.4	22.5	18.2	30.4
25–30	19.4	18.8	23.6	16.5	18.9	16.0	30.8
31–36	28.1	5.1	21.9	9.0	22.6	14.2	19.5
Field E 5							
0–6	17.5	29.6	....	25.6	....	37.0	....
7–12	43.7	40.3	....	40.4	....	40.2	....
13–18	38.3	38.5	....	36.1	....	41.1	....
19–24	37.0	23.3	....	28.4	....	32.4	....
25–30	27.9	21.5	....	22.7	....	30.8	....
31–36	24.1	3.4	....	15.7	....	32.6	....

The data presented in tables 16 and 17 indicate that the effect of lime applied to the surface in sufficient amounts decreased the unsaturation of the soils. The amount of decrease varies more or less with the individual soil. Complete saturation can be expected only when the pH is above 8.3.

Penetration of lime as shown by effect upon base complex

The effect of 8 tons of lime upon the base complex was confined to the surface 6 inches on fields E 4, E 5, E 3, and E 2 and to a depth of 2 feet on field E 1. The depth to which the lime had reached was shown in two ways, first by an increase in the calcium and magnesium content, and second by a decrease in the hydrogen content and the degree of unsaturation (tables 15, 16, and 17). This effect was apparent on the surface of all plots which received heavy applications of lime. On the sand the changes extended deeper.

Previously Rost and Fieger (25) in their work on the effect of lime on the hydrogen-ion concentration of these soils had observed the penetration of lime into the lower depths as shown by the change in the hydrogen-ion concentration. They reported that the effect of an 8-ton application of limestone extended to a depth of 12 inches upon fine-textured soils and to a depth of more than 3 feet upon the sand of field E 1. The penetration of the lime in these soils as determined by the changes in the exchange complex is not so great. It is possible, however, as a study of the data on the effect of lime will show, that in many cases as much as 2 tons of lime could penetrate into the lower depths of the soil and still not affect the exchange complex noticeably. From a comparison of the data obtained by Rost and Fieger on the hydrogen-ion concentration and that obtained in this study it would appear that the lime applied at the surface of any soil except sands has but a small effect upon the exchange complex below the depth of mechanical incorporation although it may affect the hydrogen-ion concentration to greater depth.

Buffer capacity

The effectiveness of lime as a corrective of acidity is limited by the buffer capacity of the soil. This is the inherent ability of a soil to resist changes in its hydrogen-ion concentration through the application of substances which would tend to either increase or decrease the hydrogen-ion concentration. Hoagland and Christie (10) have shown that this capacity is greater in fine textured soils than in coarse and that it will require less base to cause a definite change in reaction on a sand than on a fine textured soil.

Various explanations of this phenomenon have been advanced by investigators. The data from the five experimental fields indicate that the buffer capacity may be directly connected with and even dependent upon the base capacity of the soil. It is to be expected that 8 tons of limestone, regardless of whether it is applied upon a silty clay loam soil such as field E 4 or upon a loamy sand as in field E 1, will produce a somewhat equal concentration in the soil solution. The ratio of this concentration to the unsaturation in the base capacity of a soil such as a loamy sand is much greater than it is on a fine textured soil. In the former case, if an unsaturation of 50 per cent be assumed, the lime has only to replace about 4 m.e. of hydrogen per 100 gm. of soil, since in sandy soils used in this study the exchange capacity is rarely over 8 m.e.

In the case of a silty clay loam with an exchange capacity of 34 m.e. and a 50 per cent unsaturation, the same amount of lime would have to replace at least 17 m.e. of hydrogen per 100 gm. of soil. It should then be about four times as effective on the first soil as on the second if the lime is considered equal in its effective power in both cases.

That such may be the case appears likely from a study of the effect of the 8-ton application of lime stone upon the degree of unsaturation in the five experimental fields. The sample from field E 1 had the greatest unsaturation, 50 per cent, and field E 4 had only 43 per cent. On other fields the unsaturation was less than 50 per cent. Despite the fact that field E 4 has less unsaturation than field E 1, the unsaturation is reduced only to 33.1 per cent. This field with the highest base capacity has been the least corrected in unsaturation by an 8-ton application of ground limestone. On field E 5 with the next highest base capacity, 32 m.e., the unsaturation was reduced slightly more, or from 37 per cent to 30 per cent. This decrease is on the plot which received 4 tons of hydrated lime, the effect of this amount being equal to that of 8 tons of the finely ground limestone applied to the other fields. The base capacity on field E 3 is 27 m.e. and the degree of unsaturation was lowered by 8 tons of lime from 47 per cent of 25.5. In field E 2 which has a base capacity of 24 m.e. a similar treatment decreased the unsaturation from 39 per cent to 8.7 per cent. On field E 1, where the exchange capacity is 7.8 m.e., 8 tons of lime completely saturated the exchange complex. In table 18 are presented the fields in order of their decreasing exchange capacity. It will be seen that the unsaturation on the plots receiving 8 tons of lime is lowered least upon field E 4.

TABLE 18

Unsaturation after 8-ton application of lime and the exchange capacity of experimental fields

FIELD	EXCHANGE CAPACITY	UNSATURATION AFTER 8-TON LIME APPLIED	UNSATURATION ON A CONTROL PLOT	RANK IN BUFFER CAPACITY BY 2 PER CENT CITRIC ACID
	m.e.	*per cent H*	*per cent H*	
E 4	34.0	33.1	43.0	1
E 5	32.0	29.6*	37.0	2
E 3	27.5	25.5	47.0	4
E 2	24.0	8.7	39.0	3
E 1	7.8	0	50.0	5

* 4-ton application of hydrated lime.

Relation of unsaturation to crop response to lime

From time to time the degree of unsaturation has been suggested as a measure of lime deficiency. Recently Turner (28) working on sugar cane soils has published a report in which he suggests using the unsaturation as determined by the method of Page and Williams as a measure of the lime requirement. By this he estimates that about three times the amount of lime necessary to bring the degree of unsaturation to 30 per cent is required to produce a maximum effect. He found 30 per cent unsaturation to be the limit in his so-called "good soils." Kappen also has set this value as the approximate one at which no great response to lime may be expected.

For a study of the problem the control plots from the 28 fields were used. The properties of the soils are shown in table 2. They were selected to show as wide a variation in the response of alfalfa to lime as possible. This range was from no response on such fields as D 6 to an increase in yield of 209 per cent on D 2.

The data from this study do not indicate a close relationship between the degree of unsaturation of the soil and the response to lime (table 19). They rather indicate that the relationship is general and that it holds more nearly in the more responsive soils. That is, of those which gave a definite response to lime only 4 out of 19 had an unsaturation of less than 39 per cent, and of those above 40 per cent unsaturated 14 of 16 showed decided responses. On the other hand the least unsaturated soil in the group, field D 8, with an unsaturation of 14.6 per cent, gave what may be termed a definite response though not a large one. In the soils which did not respond markedly the correlation between the crop behavior and the unsaturation is less. Of the nine soils in this group six had unsaturation of 37 per cent or above, two had 50 per cent.

TABLE 19

Increase in yield of alfalfa from lime and degree of unsaturation on 28 fields in southeastern Minnesota

GLACIAL OUTWASH			LOESS			GLACIAL DRIFT		
Field	Increase in yield	Percentage unsaturation	Field	Increase in yield	Percentage unsaturation	Field	Increase in yield	Percentage unsaturation
	per cent			*per cent*			*per cent*	
O 2	89	44.6	L 7	207	46.6	D 2	209	51.5
O 4	37	42.5	L 8	140	48.0	D 1	98	50.5
E 1	23	50.0	L 1	105	29.5	D 4	86	46.5
O 1	11	31.2	L 6	87	44.3	E 4	59	41.0
O 5	9	51.7	E 3	76	44.0	D 7	55	37.6
O 3	0	37.9	L 5	48	31.7	D 3	36	41.6
	..	....	L 10	46	40.4	D 8	24	14.6
	..	....	L 3	36	39.1	D 5	23	45.0
	..	....	L 2	22	39.4	E 5	19	37.0
	..	....	E 2	20	39.0	D 6	2	32.7
	..	....	L 4	17	50.9		...	....
	..	....	L 9	3	21.8		...	....

To sum up, the unsaturation as an indication of crop behavior is of doubtful value on soils which show little unsaturation. Of those which show unsaturation of 40 per cent or above this study would indicate that about 85 per cent may be expected to give definite returns from lime.

SUMMARY

A study was made of the exchangeable bases in the soils of 28 fields in southeastern Minnesota. It included an investigation of the amount of replaceable bases, the degree of unsaturation, and the effect of lime upon the exchange complex and degree of unsaturation. The relationships existing between the unsaturation and the hydrogen-ion concentration and its relation to crop behavior with lime were also studied.

The soils were selected from experimental and demonstration fields of the University of Minnesota. From the demonstration fields only samples from the untreated plots were used, whereas from the experimental fields samples from plots receiving 1, 2, 3, 4, and 8 tons of lime as well as from the control plots were taken for analysis. The response of alfalfa to lime varied widely. The texture of soils ranged from loamy sand to silty clay loam.

The Gedroiz 0.05 *N* hydrochloric acid method was used to determine replaceable base, Bradfield's method using the NH_4OH—NH_4Cl buffer method for replaceable hydrogen, and the electrometric method, for the hydrogen-ion concentration.

The base capacity ranged from very low, 6.7 m.e., on the loamy sand to high, 48.0 m.e., on the silty clay loam. On all fields except the loamy sands and one silty clay loam the moisture equivalent was almost identical with the number of milliequivalents in the base capacity per 100 gm. of soil. Any marked textural change was noticeable in the base capacity.

The replaceable bases determined in these soils were calcium, magnesium, and hydrogen. The calcium was from three to six times as great as the magnesium and together they average 60 per cent of the total replaceable base. The magnesium appeared to be less affected by varying amounts of hydrogen present.

The content of replaceable base decreased from the surface downward. The decrease was about 2 m.e. of hydrogen and 2 of calcium in the second 6 inches and on soils developed on outwash and glacial drift there was a similar decrease in the second foot. On soils developed on loess there was somewhat less decrease below the first 6 inches. The magnesium remained more or less constant throughout the profile.

The degree of unsaturation in the surface soils varied widely but was generally high, averaging about 40 per cent. In most cases it was equally high in the second foot, but in the third foot there was a marked decrease.

The relationship of unsaturation to hydrogen-ion concentration was only general. Usually the more unsaturated soils had a lower pH. In the range of hydrogen-ion concentration from a pH of 5.8 to a pH of 6.2 there were wide variations in unsaturation. When the pH approached the neutral point the unsaturation was rather uniformly low.

An application of 8 tons of limestone an acre increased the total base capacity on four of the five experimental fields. Upon only one, E 2, did an amount less than 8 tons produce any increase, but on this the 4-, 3-, and 2-ton rates were effective.

Limestone increased the replaceable calcium and magnesium somewhat in proportion to the amount applied, and in most cases the increase in calcium and magnesium exceeded the decrease in replaceable hydrogen. On fine textured soils it was necessary to apply at least 3 or 4 tons of finely ground limestone before any definite effect on the exchange complex could be observed, but on the coarse textured soil the effect of 1 ton was discernible.

The lime application decreased the degree of unsaturation more or less in proportion to the amount applied. On the loamy sand 8 tons of limestone caused complete saturation with a pH of 8.3.

The penetration of lime as observed by its effect upon the replaceable bases was not detected below the surface 6 inches except on the loamy sand. There it appears to have reached to the lower part of the second foot.

The buffer capacity of these soils was closely correlated with the exchange capacity. More lime was required to produce an effect on fine textured soils with high capacity than to produce an equal effect on coarse textured soils with a low exchange capacity.

The use of the degree of unsaturation as an indication of the crop response to be expected from lime is of doubtful value. If the soil is more than 40 per cent unsaturated the chances are that there will be an increase from liming in about 85 per cent of the cases. However there were a considerable number of soils having rather low unsaturations which gave quite as great a response to lime.

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EFFECTS OF STABLE MANURE AND CERTAIN FERTILIZERS ON THE MICROBIOLOGICAL ACTIVITIES IN VIRGIN PEAT[1]

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Better results have often been obtained by applications of animal manures to various types of virgin peat soils than by addition of like amounts of the same available plant-foods in the form of commercial fertilizers. This fact has favored the viewpoint held by those who maintain that the natural microbial activity in peat is low or totally absent and the most valuable effect of stable manure on peat is due to the microbes in the manure. Waksman's (17) review of the literature on the occurrence and abundance of microörganisms in peat bogs and his own work in this connection afford conclusive evidence that the microörganisms naturally present in peat soils play an important part in the transformation and liberation of plant-food. Those who maintain that the introduction of the bacterial flora of stable manure brings about greatly changed microbiological activities in soil have never offered really convincing proof of their contention. On the other hand, the many unsuccessful attempts to change soil flora by additions of cultures of certain microörganisms, or mixtures thereof, are evidence against such claims.

According to Tacke (12) the addition of stable manure to lowmoor peat has a favorable effect in promoting the liberation of available plant-food. Waksman (17) ascribes this effect to the increase in available carbon compounds with a suggestion that it might be due also to the microflora in the manure. That the liberation of plant-food in certain virgin peats supporting the growth of grasses, and therefore seemingly well supplied with available carbon compounds, is sometimes greatly stimulated by the addition of stable manure suggests a beneficial effect from the microflora introduced by the manure. However, Barthel and Bengtsson (1) concluded that microörganisms added with the manure are of no practical importance in normal mineral soils, since in their work sterilized manure acted in the same manner as unsterilized manure. In view of the divergence of opinion regarding this subject the work presented in this paper was undertaken in an attempt to ascertain the effect, if any, of the microflora of stable manure and also the effect of stable manure, wheat straw, and certain fertilizers on the microbiological activities in a virgin peat soil.

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EXPERIMENTAL PROCEDURE

The peat for this investigation was obtained from a virgin area near Everett, Washington, in T 29N, R 5E., Section 22, adjacent to a cultivated field in the same bog. It is a poorly decomposed, woody-sedge peat, reclaimed by diking about 15 years ago, and resembling that of the Monroe type described by Dachnowski-Stokes (4). Its reaction determined by means of the quinhydrone electrode in a suspension of one part of peat to five parts of distilled water was pH 4.3. Qualitative determinations for available plant-food in the surface 8-inch layer revealed only a trace of phosphorus with 0.2 N nitric acid and showed that this layer is low in replaceable calcium and potassium as determined by means of neutral salt replacement. Crop yields on the unfertilized peat under cultivation are poor and unprofitable but may be increased many times by applications of manure or phosphate fertilizers. Applications of nitrogen or potash fertilizers alone or in addition to phosphate fertilizers do not result in any marked increase in yield. It is evident that phosphorus is the limiting plant-food for crop production and must be supplied to this soil to obtain good crop growth.

In the fall of 1929 a representative quantity of this virgin peat taken from the surface 8-inch layer in the field was shipped to the laboratory, air-dried sufficiently to permit pulverization by rubbing, passed through a 16-mesh sieve, and thoroughly mixed. Under these conditions the moisture content was 18.1 per cent whereas that of the completely air-dried peat was 7.65 per cent. Unnecessary drying was avoided in order to prevent destruction of the microbial flora as much as possible. Water was added to the pulverized peat to make its moisture content equivalent to that of the field soil, which was approximately 200 per cent at optimum conditions for tillage operations and plant growth. A preliminary determination with a small sample of the pulverized material showed its water holding capacity had decreased as a result of drying and pulverization and it was found necessary to adjust its moisture content to 180 per cent instead of 200 per cent to obtain optimum moisture conditions. Following the addition of moisture the soil was allowed to stand for one week to assure uniform moisture distribution and as far as possible the normal resumption of the microbiological activities. For the first experiment six portions, each consisting of 1,000 gm. of moist peat, were thoroughly mixed with the various ingredients added for experimental purposes and were placed in wide-mouth bottles connected with an absorption train for carbon dioxide determinations. The carbon dioxide evolution was determined daily during the first week when microbiological action was greatest and less frequently thereafter, the interval between determinations being gradually increased until it was seven days beginning with the fourth week. Nitrate nitrogen, ammonia, and the number of fungi, actinomyces, cellulose decomposing bacteria, Azotobacter, and total bacteria were determined periodically. The moisture content of the peat was maintained at 180 per cent by addition of water when necessary. The treatment of the various lots of peat was as follows:

Number of sample	*Treatment per gm. 1,000 moist peat*
1	Check
2	0.7 gm. superphosphate(16.5 per cent)
3	7 gm. manure (air-dried)
4	7 gm. manure plus 0.7 gm. superphosphate
5	7 gm. sterilized manure plus 0.7 gm. superphosphate
6	7 gm. wheat straw plus 0.7 gm. superphosphate

The rate of application of these materials was equivalent to 600 pounds superphosphate, 10 tons of manure, and 3 tons of straw per acre based on the acre volume of the surface 7 inches of peat.

The manure was mediumly decomposed stable manure containing 2.04 per cent nitrogen on the dry basis. That used for sample 5 was sterilized in the autoclave at 15 pounds pressure for 2 hours on two successive days. Sufficient sodium nitrate was added to the finely ground wheat straw to make its nitrogen content equal to that of the manure.

At the end of 68 days the peat soil in all the bottles was inoculated with 25 cc. of a 1:5 suspension of Palouse silt loam known to contain an abundance of nitrifying bacteria and Azotobacter as well as other soil microbes. This was done to ascertain the effect of inoculation with the microflora of a fertile mineral soil on various microbiological activities in peat.

At the end of 129 days a second experiment was started to determine the effect of lime on nitrification and other microbiological activities in these variously treated peats. Because frequent sampling during the first experiment had decreased the volume of the soil in the bottles to a considerable extent it was considered advisable to combine the contents of two bottles into one previous to making lime applications. The arrangement was as follows:

Number of new samples	*Number of old samples combined*	*Treatment*
1	1 and 2	2 per cent $CaCO_3$ on dry peat basis
2	2 and 4	check
3	5 and 6	2 per cent $CaCO_3$ on dry peat basis

The calcium carbonate was thoroughly mixed with the peat, the latter being placed in the bottles, which were connected with a CO_2 collection train, and the same determinations were made as in the first experiment.

METHODS OF ANALYSIS

Sampling

Samples of peat for analysis were obtained by passing the contents of each bottle through a 6-mesh screen on a large cloth, mixing further by rolling the soil back and forth on the cloth before removing the required quantity for analysis. This method insures thoroughly mixed, representative samples, and at the same time, prevents the tendency toward possible anaerobic conditions developing in the lower part of the bottles. After the samples were removed, the peat was returned to the bottles and packed by gently tapping the bottles on a table. The samples for the various analyses were spread evenly in a thin layer on paper and air-dried in the laboratory. This process of drying required only a few hours. All determinations were made on air-dry peat and the results calculated on the basis of dry peat.

Microbiological analysis

The direct microscopic method of Conn (3) was adopted for the determination of the total number of bacteria. Fungus spores and mycelia were not included in the counts.

The numbers of fungi, actinomyces, cellulose decomposing bacteria, and Azotobacter were determined as separate groups by plating on selective culture media. The fungi and actinomyces were determined from soil suspensions by the dilution plate method. Counts were made on duplicate plates and the numbers averaged for computation. The nutrient medium for fungi was potato extract agar adjusted to approximately pH 3.2 to 3.4 by addition of sterilized 0.1 *N* citric acid to the agar medium at the time the plates were poured. The actinomyces were grown on Waksman's (13) albuminate agar adjusted to pH 8. Silica gel plates 15 cm. in diameter were prepared as a substratum for the growth of Azotobacter and cellulose decomposing bacteria according to the method of Waksman (14). No duplicate plates were used for these organisms. Winogradsky's (18) nutrient solution for the growth of Azotobacter was used according to his direction, and Waksman's (14) nutrient solution and a sheet of filter paper superimposed upon the silica gel were used for the development of cellulose decomposing bacteria. The air-dried peat was spread evenly over the surface of these plates at the rate of 300 gm. per plate. All plates were incubated at 27°C. and the colonies of the respective groups of microörganisms counted at the end of the incubation period.

Chemical analysis

Total nitrogen was determined by the Kjeldahl method. Ammonia and nitrate nitrogen were determined on water extracts, nitrate nitrogen by the phenol-disulfonic acid method, and ammonia nitrogen by Nesslerization according to the official and tentative methods of the Association of Official Agricultural Chemists (8). The carbon dioxide collected in the NaOH solution of the absorption train was determined according to the method of Heck (7). Total carbon was determined by wet combustion using the method of Friedemann and Kendall (5). Unless stated otherwise the pH was determined by means of the quinhydrone electrode in a suspension of one part of soil in five parts of distilled water.

EXPERIMENTAL RESULTS

Before the different portions of peat were mixed with the various ingredients and placed in bottles the separate groups of microörganisms, carbon, nitrogen, and reaction were determined on the moist peat and on the air-dried unsterilized manure in the manner described in the foregoing. The results of these determinations are given in table 1.

It is interesting to note that Azotobacter were present in this peat although its reaction was pH 4.3, which is much more acid than the arbitrary limits set by Gainey (6), who concluded that very few soils more acid than pH 6 contain Azotobacter. To make sure that their presence in this soil was not due to accidental contamination separate, fresh samples from the field were examined and similar results obtained. Unpublished data obtained in this laboratory show that the occurrence of small numbers of azotobacter in western Washington soils that are more acid than pH 5.5 is not unusual. Whether the activity of the Azotobacter in these acid soils has any practical significance remains to be determined, but the fact that they are present under these adverse conditions is not without interest.

TABLE 1

Determinations on original peat and manure based on dry material

DETERMINATION	PEAT	MANURE
Bacteria per gram	769,537,000	3,747,239,000
Fungi per gram	1,871,000	20,000
Actinomyces per gram	2,760	640,000
Cellulose decomposing bacteria per gram	92	350
Azotobacter per gram	18	None
Per cent N	1.93	2.04
Per cent organic C	37.10	37.34
P.p.m. NO_3	116.00	35.10
P.p.m. NH_3	243.30	Trace
pH	4.3	8.4

There was an abundance of microörganisms in the moist peat after it had been allowed to stand for one week, showing that microörganic life was active. Probably the drying and subsequent wetting of the soil had caused a temporary stimulation of the general microbiological activities and especially of the development of bacteria, since the latter suffered a sudden reduction soon after the experiment was started as will be noted later. In this connection it may be recalled that the bacteria were determined by direct microscopic counts, and therefore, their number would be expected to be considerably larger than if they had been determined by the plate method, for colonies counted on plates may result from groups of cells as well as from single cells. Furthermore, not all cells develop in the media used, because no one nutrient medium has ever been invented that is suitable for growth of all the different species of bacteria in the soil. Although results obtained by direct microscopic counts are not entirely free from criticism, nevertheless, they probably give a more accurate index of the actual number of bacteria in soil than do plate counts.

It is noteworthy to observe the impressive number of bacteria and the abundance of actinomyces and fungi in the air-dried, unsterilized manure. The rate of application of this manure was equal to 1.93 per cent of the dry weight of peat. Thus the actual numbers of the various microörganisms added to the peat by means of unsterilized manure comprised approximately 73,460,870 bacteria, 392 fungi, and 12,389 actinomyces per gram of dry peat. This is an appreciable increase which should be reflected in subsequent microbiological analyses during the course of the experiment if the introduction of the microflora of the manure has any marked effect upon the microbiological activities in the peat.

TABLE 2

Carbon dioxide evolution

Expressed in mgm. of carbon per 100 gm. dry peat

DAYS	SAMPLE 1	SAMPLE 2	SAMPLE 3	SAMPLE 4	SAMPLE 5	SAMPLE 6
1	10.5	9.5	17.0	14.7	21.5	22.2
2	12.7	13.3	19.8	19.5	23.0	28.2
3	11.4	10.3	12.8	14.8	14.3	19.7
4	9.7	9.3	12.5	13.3	13.9	15.8
5	10.9	11.9	12.8	12.9	11.2	12.9
6	9.8	9.3	11.3	11.2	11.8	12.5
8	18.7	19.5	22.7	23.5	24.8	24.7
11	29.5	30.7	36.3	38.0	39.4	39.8
14	25.5	27.0	32.8	32.2	33.5	33.3
19	36.8	44.4	44.2	46.7	45.3	40.3
26	48.3	58.8	62.0	67.5	65.5	60.8
33	34.1	36.8	38.2	37.5	37.2	39.4
40	37.3	44.0	55.5	53.7	52.7	59.0
47	47.5	55.8	64.3	68.2	64.3	90.3
54	45.8	48.3	64.6	68.0	54.8	82.5
61	16.8	39.2	64.0	59.8	51.0	63.5
68	39.4	36.5	61.4	48.9	45.8	44.8
75	36.4	33.6	56.0	39.6	40.4	31.3
82	30.2	29.4	47.6	38.3	33.9	26.3
89	30.8	40.4	47.9	38.1	28.4	27.6
103	37.6	35.7	53.9	40.6	47.5	35.7
Total.....	579.7	643.7	837.6	787.0	760.2	810.6

Experiment 1

To acquire a more comprehensive conception of some of the changes taking place in the peat as a result of the various treatments the author measured the total microbiological activities by the carbon evolved as carbon dioxide as well as by the changes in ammonia and nitrate nitrogen and the changes in numbers of the various groups of microörganisms as specified in the experimental procedure. Most of these changes were expected to take place most rapidly during the early part of the experiment, at least in the soils to which organic residues had been applied. For this reason carbon dioxide determinations and microbiological analyses were made frequently during this period and less frequently later. The results of the carbon dioxide determinations are given in table 2. From a consideration of the total amounts produced by the variously treated peats it may be noted that the application of superphosphate to sample 2 stimulated the evolution of CO_2 slightly, whereas the application of manure to samples 3, 4, and 5 and of wheat straw to sample 6 caused a marked increase in CO_2 production. As would be expected, this increase was greatest during the first dew days of the experiment, as represented graphically in figure

TABLE 3

Total bacteria per gram of dry peat

(Last three figures omitted)

DAYS	SAMPLE 1	SAMPLE 2	SAMPLE 3	SAMPLE 4	SAMPLE 5	SAMPLE 6
0	769,537	769,537	769,537	769,537	769,537	769,537
4	263,562	405,105	745,133	587,321	496,213	322,132
8	405,105	388,835	377,447	328,640	427,882	271,697
26	514,109	614,979	383,955	369,313	483,198	601,963
61	569,425	458,793	361,177	436,017	515,736	357,924
103	571,051	509,228	358,660	383,955	649,144	439,271
Average...	464,504	475,388	445,274	421,049	514,435	398,597

TABLE 4

Fungi and actinomyces per gram of dry peat

(Last three figures for fungi numbers omitted)

DAYS	FUNGI IN SAMPLES						ACTINOMYCES IN SAMPLES					
	1	2	3	4	5	6	1	2	3	4	5	6
0	1,871	1,871	1,871	1,871	1,871	1,871	2,760	2,760	2,760	2,760	2,760	2,760
4	6,992	4,600	6,348	7,452	7,636	3,864	9,200	5,520	9,200	3,680	7,360	10,120
8	4,048	3,220	5,152	2,024	4,140	3,036	13,800	8,280	11,040	11,960	4,600	15,640
14	7,084	1,978	2,539	1,297	4,784	.....	276	920	1,012	......	736	644
19	3,680	3,772	1,656	1,306	1,417	1,536	1,012	1,196	1,288	1,656	3,036	1,656
26	1,481	1,886	1,306	1,444	1,260	1,564	3,220	4,784	4,232	3,864	2,208	......
33	2,475	1,914	1,904	2,107	2,061	1,950	3,772	4,048	7,084	5,428	1,840	1,012
40	2,079	1,343	1,592	1,150	1,205	1,205	2,668	6,164	7,084	4,324	3,404	7,820
47	1,960	1,573	1,628	1,665	1,334	1,766	4,968	2,576	2,300	3,680	2,116	3,956
54	2,042	2,355	1,518	1,509	2,052	1,233	9,016	6,164	13,892	7,728	9,660	6,256
61	1,463	1,619	994	736	1,380	1,325	13,892	10,488	8,740	......	12,880	10,672
68	1,527	1,776	1,352	1,417	1,444	828	7,360	18,216	16,744	6,256	15,824	6,624
75	1,408	764	1,021	837	1,187	828	10,396	6,256	4,600	6,532	13,984	6,900
82	1,592	1,104	1,978	1,196	883	1,822	7,636	6,900	12,144	3,496	10,672	9,936
89	1,122	1,058	1,527	782	948	1,923	10,304	7,820	8,188	3,864	7,176	9,660
103	1,187	938	322	460	644	1,095	......	......	......	......	......	......
Average..	2,676	1,993	2,056	1,692	2,158	1,713	6,966	6,381	7,682	5,206	6,821	6,992

2 for treatment 6. Although the shape of the carbon curve would be similar for all treatments, the high points in the curve would be considerably lower in treatments 1 and 2 where no manure or straw was applied than in the samples that had received manure or straw. Available carbon and nitrogen compounds appeared to be the controlling factors, since the amount of CO_2 produced in the peat treated with straw compared favorably with that of the peats treated with stable manure. No significant difference in CO_2 production existed as a result of sterilization of the manure.

The numbers of total bacteria, fungi, actinomyces, cellulose decomposing bacteria, and Azotobacter determined at each sampling are given in Tables 3, 4, and 5. The most striking feature in these data is the sudden decline in numbers of bacteria in contrast with an equally sudden increase in numbers of fungi and actinomyces during the first 4 days of the experiment. The numbers of fungi remained relatively high for 2 weeks and gradually declined thereafter. The rapid increase in numbers of actinomyces continued for 8 days but was followed by a sudden drop and at the end of 3 weeks by a general but fluctuating rise reaching a second high peak at approximately 60 days. The numbers of cellulose decomposing bacteria and especially of Azotobacter were too small to have any significant bearing upon the results.

TABLE 5

Cellulose decomposing bacteria and azotobacter per gram of dry peat

DAYS	CELLULOSE DECOMPOSING BACTERIA IN SAMPLES						AZOTOBACTER IN SAMPLES					
	1	2	3	4	5	6	1	2	3	4	5	6
0	92	92	92	92	92	92	18	18	18	18	18	18
4	0	46	202	83	0	9	..	..	..	..	..	..
8	0	64	55	74	28	55	..	..	..	..	..	..
14	9	28	0	55	18	92	9	18	9	9	18	9
19	202	110	92	55	0	175	..	..	..	..	..	..
26	37	18	110	147	129	18	..	..	..	..	..	..
33	0	28	83	193	166	0	..	..	..	..	..	..
40	37	83	74	166	156	0	..	..	..	..	..	..
47	55	23	74	51	97	92	..	..	..	..	..	..
54	14	23	28	14	28	32	..	..	..	..	..	..
61	110	147	74	156	239	9	..	..	..	..	..	..
68	230	74	120	386	175	46	..	..	..	..	..	..
75	9	37	55	92	74	18	0	9	9	9	18	0
82	83	74	83	92	46	46	..	..	..	..	..	..
89	37	28	37	55	92	74	28	0	0	0	0	28
103	...	...	...	...	...	...	0	0	37	0	0	28
Average...	59	56	78	116	89	48	11	9	15	7	11	17

Apparently the large number of bacteria present at the time the experiment was started was the result of an abundant supply of readily available nutrients brought about partially by pulverization, but more especially by the drying and subsequent wetting of the peat. This excessive food supply was soon exhausted, thereby causing the sudden drop in numbers of bacteria. The fungi and actinomyces, which are known to be capable of utilizing the more complex carbonaceous compounds to secure their energy, were not affected by the depletion of the readily available nutrients but were abundantly supplied with food suitable for their needs and therefore multiplied rapidly in proportion to the amount of food available. These results make it appear that the bacterial flora in this peat was able to compete successfully with the fungi and actinomyces as long as the readily available nutrients were present in abundance, and lead to the suggestion that bacteria as well as fungi take a prominent part in the decomposition of the readily available carbonaceous compounds in the organic matter.

Judging from the number of bacteria found on the fourth day of the experiment it seems that relatively few of the 73,460,870 bacteria added per gram of dry matter in samples 3 and 4 by means of unsterilized stable manure had survived, and even this effect was only temporary in nature, as in subsequent determinations the number of bacteria in these two samples and in the others was similar. A general analysis of the data relative to separate groups of microörganisms at each determination and comparison of these with the average number of each group throughout the experiment and with the total amount of carbon dioxide evolved as a result of each treatment clearly indicate that the microflora in the stable manure added to this virgin woody-sedge peat had no significant effect upon either the number of microörganisms or their activities. The results obtained from the samples treated with unsterilized manure, sterilized manure, and wheat straw in addition to superphosphate are comparable, as may be seen in figure 1. Naturally, the decomposition of wheat straw and manure could hardly be expected to proceed in exactly the same manner because there are distinct differences in the composition of the nitrogenous and carbonaceous compounds in the two substances. According to Waksman and Diehm (15), the chemical complexes of the animal excreta, which usually make up a large part of stable manure, have been modified materially by passage through the digestive tract of animals and therefore the excreta have been enriched in lignins and proteins. It is shown in figure 1 that the numbers of bacteria, and fungi, at the beginning of the experiment were lowest in the straw treatment. The average numbers of bacteria as given in table 3 for these three samples was also lowest in the straw treatment, but the average number of actinomyces and the total microbiological activities as measured by the liberation of CO_2 were greatest, so that the sum total of the microbiological activities for these three treatments may be considered to be comparable. Evidently the effect produced by these organic residues was due to their available carbon and nitrogen compounds. Barthel and Bengtsson's (1) results obtained from the addition of unsterilized and sterilized stable manure to mineral soil and those obtained here are similar, and their contention that the microörganisms added with the manure are of no practical importance in the decomposition of organic matter in normal soils was substantiated in this experiment in so far as virgin peat is concerned.

Nitrate nitrogen and ammonia were determined regularly at each sampling and the results are summarized in table 6. It may be observed that with the

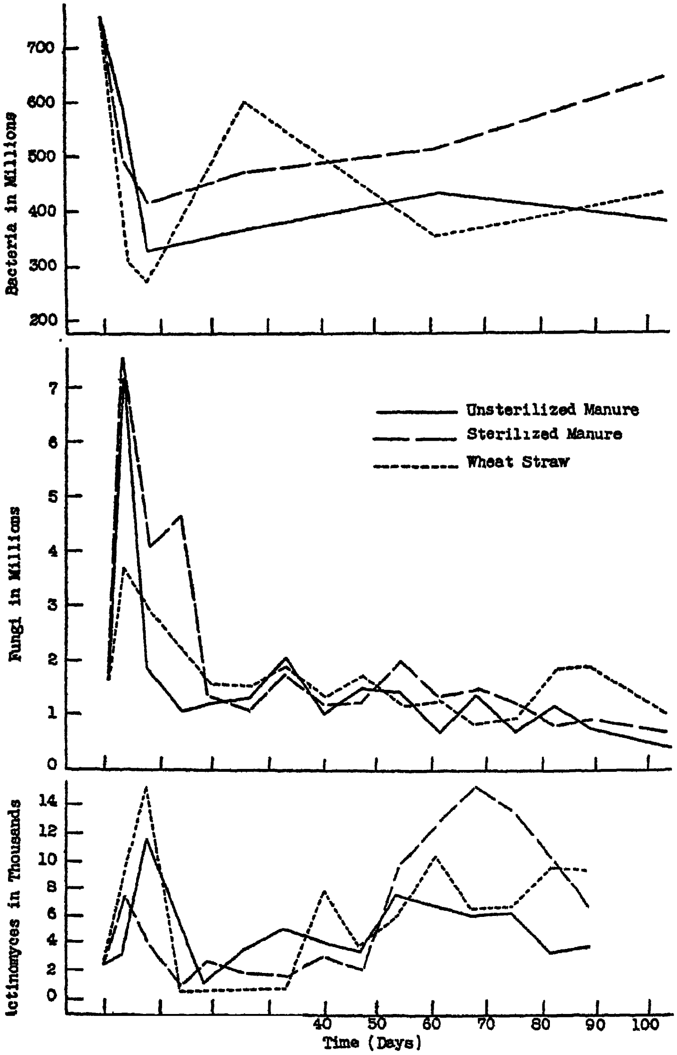

FIG. 1. BACTERIA, FUNGI, AND ACTINOMYCES IN PEAT TREATED WITH UNSTERILIZED MANURE, STERILIZED MANURE, AND WHEAT STRAW IN ADDITION TO SUPERPHOSPHATE

possible exception of sample 6, nitrification was rather slow. The maximum amount of nitrate nitrogen accumulated at any one time was 80 parts NO_3 per million parts of dry peat whereas the average accumulation of NO_3 during the entire course of the experiment was only 20 p.p.m. The results of Sievers and Holtz (11) show that under similar conditions the accumulation of nitrate nitrogen in fallow, mineral soil containing less than one-tenth as much total nitrogen as the peat was approximately twice the amount liberated in peat during the same period of time. This indicates that nitrification in the peat soil proceeded slowly considering the abundance of ammonia accumulated and, therefore, available at all times for nitrification by the nitrifying bacteria. The exception in sample 6 may be accounted for in part at least by the nitrate nitrogen added with the straw, as its original total nitrogen content, which was 0.25 per cent, was increased to equal that of the manure by addition of $NaNO_3$. Since certain types of microörganisms are able to carry on their activities with ammonia as a source of nitrogen, it is likely that only a part of the nitrate nitrogen added with the straw was required for the microbiological activities and the remainder was recovered in subsequent analyses.

TABLE 6

NO_3 and NH_3 in p.p.m. based on dry peat

DAYS	SAMPLE 1		SAMPLE 2		SAMPLE 3		SAMPLE 4		SAMPLE 5		SAMPLE 6	
	NO_3	NH_3	NO_3	NH_3	NO_3	NH_3	NO_3	NH_3	NO_3	NH_3	NO_3	NH_3
0	94.3	197.8	94.3	197.8	94.3	197.8	94.3	197.8	94.3	197.8	94.3	197.8
4	99.8	259.7	108.1	249.5	97.0	187.6	83.2	187.6	97.0	218.1	266.2	187.6
8	33.0	196.5	37.2	196.5	37.2	186.4	45.5	206.6	35.8	175.4	137.7	180.9
14	85.1	166.8	106.4	166.8	79.8	166.8	79.8	155.2	95.7	161.9	319.1	161.9
19	169.2	269.2	143.6	288.0	174.4	288.0	160.0	280.8	150.0	280.8	400.0	327.4
26	132.7	49.8	172.6	137.2	146.0	158.2	132.7	149.3	139.4	141.6	398.2	141.6
33	142.7	280.9	129.1	203.9	129.1	267.3	129.1	280.9	122.3	280.9	353.3	229.9
40	147.7	302.0	134.2	302.0	134.2	335.6	134.2	302.0	134.2	302.0	362.4	318.8
47	140.1	382.2	140.1	406.6	133.8	238.9	133.8	274.9	133.8	251.6	369.4	238.9
54	132.1	260.0	132.1	283.0	132.1	271.5	119.5	307.1	122.6	271.5	339.6	235.9
61	133.3	272.7	157.6	318.2	145.5	272.7	151.5	272.7	157.6	296.0	424.2	250.5
68	109.8	343.5	109.8	274.4	115.8	252.0	109.8	297.8	115.8	274.4	341.5	182.9
75	107.0	424.5	113.2	401.5	107.0	471.7	119.5	471.7	116.3	354.3	301.9	388.9
82	90.0	217.6	90.0	144.7	90.0	265.8	96.5	252.9	99.7	241.2	283.0	217.6
89	100.3	376.2	94.0	282.1	94.0	282.1	87.8	235.1	99.2	212.1	288.4	188.1

The addition of either sterilized or unsterilized manure and of superphosphate did not result in any appreciable increase in accumulated nitrate nitrogen. Unless some indirect effect on nitrification occurred following the introduction of the microflora of the manure, accumulation of nitrate nitrogen as a result of the addition of the manure or of the wheat straw could hardly be expected during the course of the experiment, for, according to the work of Sievers and Holtz (10), applications of organic residues containing less than 2.25 per cent nitrogen to soil do not result in nitrate nitrogen accumulation during a similar period of time. The fact that large quantities of ammonia were present in the peat at all times regardless of treatment suggests that the slow rate of nitrification was due either to the relative inactivity of the nitrifying bacteria or to a deficiency in their number. It will be recalled that on the sixty-eighth day of the experiment 25 cc. of a 1:5 suspension of Palouse silt loam known to contain an abundance of nitrifying bacteria was added to each bottle. This inoculation failed to stimulate nitrification, as may be concluded from the data in table 6. In fact, an actual depression in accumulation of nitrate nitrogen occurred toward the end of the experiment but this was probably due to dentrification. Although the inoculation of the peat with a soil suspension introduced numerous microörganisms other than nitrifying bacteria, the microbiological activities in terms of CO_2 evolution or numbers in the various groups of microörganisms were not affected.

Waksman and Stevens (16) called attention to the fact that in the decomposition of ordinary soil organic matter the ratio of carbon liberated as CO_2 to nitrate nitrogen accumulated as nitrate is about 10:1. In their work with Everglade peats they found this ratio to be entirely different, or 1.3:1 for sedementary peat, showing that in peat soil there is a rapid accumulation of nitrate nitrogen in comparison with the amount of carbon liberated as CO_2. In later work the same authors (17) observed that different ratios existed in different peats, some of them much wider than the one suggested for ordinary soils. The ratio between carbon evolved as CO_2 and nitrogen accumulated as NO_3 and NH_3 in woody-sedge peat in this experiment fluctuated considerably at various times, but at the end of the experiment proved to be about 17:1 in the untreated peat, 33:1 in the peat treated with unsterilized manure, and 37:1 in the peat treated with straw. The addition of sodium nitrate to sample 6 did not alter this relationship to any marked extent. This shows that available nitrogen probably was not deficient in this soil, but that the carbonaceous compounds were either resistant to decomposition or the reaction and possibly the calcium content of the soil were unfavorable for the promotion of rapid decomposition of these compounds. When large amounts of carbonaceous plant residues low in nitrogen are applied to soils likewise low in nitrogen very little or no accumulation of soluble nitrogen occurs until the decomposition of the carbonaceous organic residue is well advanced. Therefore, it appears the ratio between carbon evolved as CO_2 and soluble nitrogen accumulated in the soil is a variable function changing with the nature and quantity of carbonaceous compounds as well as with the nature and quantity of the nitrogenous compounds in the soil, other things being equal. For this reason Bedford's (2) computation of microbial activities in soil on the basis of organic matter content is not applicable in all cases. It would seem that the degree of inverse relation he observed between the number of microbes per gram of organic matter and the percentage of organic matter in the soil depends upon the nature of its carbonaceous compounds as well as upon the amount of organic matter. Likewise the inverse relationship he found between bacterial numbers per gram of organic matter and the total organic matter in a given quantity of soil is not strictly applicable unless it is correlated with the nature of the organic matter.

Attention has already been called to the fact that crop production in this woody-sedge peat is poor unless some available phosphorus is supplied by means of manure or commercial fertilizer. It might be presumed that since crops respond so generously to the addition of available phosphorus, microorganisms might react in a similar manner. In the peat treated with superphosphate there was only a slight increase in the liberation of carbon as CO_2, and no perceptible beneficial effect on nitrification or on the number of microorganisms in the various groups was observed. Since it is possible that soil acidity was adverse to normal microbiological activity a separate experiment was designed to throw more light on this point.

TABLE 7

Carbon dioxide and total numbers of bacteria in peat

(Last three figures for bacteria numbers omitted)

DAYS	C PER 100 GM. DRY PEAT IN SAMPLES			BACTERIA PER GRAM DRY PEAT IN SAMPLES		
	1	2	3	1	2	3
	mgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm*
0	.....	.....	.....	292,853	297,728	309,116
7	370.0	50.7	358.9	395,343	358,016	395,343
14	138.7	51.3	121.1	432,854	351,416	476,690
21	151.9	57.5	146.4	....	....	....
28	116.8	57.0	114.5	525,590	325,386	411,613
42	216.8	98.6	211.1	....	....	....
57	267.5	49.3	274.0	540,139	356,297	505,974
79	273.2	112.5	287.2	....	....	....
106	285.8	110.6	313.5	532.005	289,593	587,321
Total.........	1,820.7	587.5	1,826.7	453,131*	329,739*	447,676*
Carbonate C...	90.0	0.0	91.5	....	....	....
Organic C.....	1,730.7	587.5	1,735.2	....	....	....

* Average.

Experiment 2

The methods employed in this experiment were essentially the same as in the previous one and the object was primarily to ascertain the effect of lime on nitrification and reaction, and also on the microbiological activities previously considered. As stated in the experimental procedure the contents of two bottles were combined into one, making three portions, two of which received 2 per cent $CaCO_3$ and one of which, sample 2, served as a control. To make sure that an abundance of nitrifying bacteria and other members of the microflora of normal mineral soil be present, each portion of peat was reinoculated with 25 cc. of a 1:5 suspension of a fertile Palouse silt loam. The results of the experiment are reported in tables 7, 8, and 9. Attention is called to the fact that 20 days elapsed between the two experiments, and during that time the numbers of bacteria and fungi had decreased considerably. This may have been partly because of a reduction in moisture content, although in experiments of this nature when no additional organic matter is supplied a gradual decline in numbers of microörganisms and a proportional reduction in the liberation of CO_2 usually occur as time progresses. That this actually took place in the woody-sedge peat may be noted by comparing the average numbers in the various groups of microörganisms and the total amounts of CO_2 liberated in

TABLE 8

Fungi and actinomyces per gram dry peat

(Last three figures for fungi numbers omitted)

DAYS	FUNGI IN SAMPLES			ACTINOMYCES IN SAMPLES		
	1	2	3	1	2	3
0	616	212	442	3,680	2,760	4,600
7	672	460	432	21,160	13,800	17,480
14	552	1,288	460	28,520	8,280	26,680
21	552	92	653	30,360	5,520	22,080
28	580	3,036	497	20,240	5,520	21,160
42	920	1,748	368	12,880	4,600	14,720
57	736	1,656	350	7,360	3,680	11,040
79	847	1,472	405	10,120	4,600	12,880
106	773	1,196	350	13,800	6,440	14,720
Average...	694	1,240	440	16,458	6,133	16,151

TABLE 9

Cellulose decomposing bacteria, azotobacter, NO_3 and NH_3 and reaction of peat

DAYS	CELLULOSE DECOMPOSING BACTERIA PER GRAM DRY PEAT IN SAMPLES			AZOTOBACTER PER GRAM DRY PEAT IN SAMPLES			NO_3 AND NH_3 IN SAMPLES						pH—QUINHYDRON ELECTRODE IN SAMPLES			
							1		2		3					
	1	2	3	1	2	3	NO_3	NH_3	NO_3	NH_3	NO_3	NH_3	1	2	3	Original peat
							p.p.m	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*				
0	14	14	14	18	18	9	131.5	65.0	280 0	57.0	168.0	64.0	4.3	4.5	4.4	4.3
7	9	14	5	74	9	46	.....	....	.....	.....	.....	.....	...	...	...	...
14	14	0	18	83	28	55	460.0	49.0	560.0	76.5	380.0	51.8	...	...	...	...
21	37	18	28	64	55	83	.....	....	.....	.....	.....	.....	...	...	...	....
28	74	41	87	37	46	74	.....	....	.....	.....	.....	.....	...	...	...	...
42	106	51	129	18	37	32	.....	....	.....	.....	.....	.....	...	...	...	...
57	78	37	133	28	0	51	.....	....	.....	.....	.....	.....	...	...	...	...
79	87	51	152	18	0	64	.....	....	.....	.....	.....	.....	...	...	...	...
106	97	37	124	32	0	74	833.5	17.4	611.9	551.4	940.1	110.0	6.6	6.3	6.7	6.5
Average...	57	31	85	41	21	54	.....	....	.....	.....	.....	.....	...	...	...	...

the untreated soil of the first experiment with those of the control soil in the second experiment.

In calculating the total amount of carbon liberated as CO_2 in table 7 the carbonate carbon in the lime was determined in the limed soils and the amount of this carbon evolved during the experiment was deducted from the totals. It is shown that the addition of lime to the peat caused a three-fold increase in the amount of carbon liberated as CO_2. Not all of this increase can be accounted for by increased numbers of microörganisms. Although liming resulted in nearly a threefold increase in numbers of actinomyces and in a marked increase in total bacteria the number of fungi was reduced approximately 50 per cent. The total average numbers of microörganisms comprising the various aerobic groups that were active in organic matter decomposition were actually less in the limed soils than in the untreated peat in the first experiment. If numbers of microörganisms as determined by the methods employed are a dependable criterion their activities as measured by CO_2 evolution must vary considerably according to environment. Evidently the addition of lime to woody-sedge peat stimulated the microbiological activities much more than the increase in numbers of microbes. Apparently the lime increased the working capacity of individual organisms and possibly this action was more favorable for the actinomyces and bacteria than for the fungi. This being the case it may be suspected that the major part of the increase in amount of carbon liberated was a result of the activity of actinomyces and bacteria, and not of fungi, as has been supposed generally.

Liming also increased nitrification materially but caused a decrease in the accumulation of NO_3 and NH_3 combined. More nitrate nitrogen and less ammonia nitrogen accumulated in the limed soils than in the control but the increase in the amounts of NO_3 was not equivalent to the decrease in the amounts of NH_3 thus pointing to the possibility that only a part of the accumulated NH_3 in the limed soils was nitrified and some of it was lost by volatilization.

The increased microbiological activity resulting from the application of lime caused a very marked change in the ratio of carbon evolved as CO_2 to soluble nitrogen accumulated. It will be remembered that in the first experiment this ratio was 17:1 for the untreated, 33:1 for the manured, and 37:1 for the straw treated peat. Sample 2 in the present experiment is comparable with the manured peats in the first experiment but its ratio of carbon evolved to soluble nitrogen accumulated is 19.7:1. That of the limed soils is 85:1 and 57:1 for samples 1 and 3 respectively. By increasing the availability of the carbonaceous compounds the ratio of carbon evolved to soluble nitrogen accumulated was widened beyond recognition, showing the extreme instability of this relationship.

The addition of lime did not affect the number of cellulose decomposing bacteria. Apparently woody-sedge peat is not a suitable medium for their development as their numbers remained small throughout the experiment.

Azotobacter seemed to be benefited by lime as their number, though small, seemed to remain rather constant in the limed soils. It is apparent that the inoculation with the Palouse silt loam suspension increased the Azotobacter population of the peat but as may be noted from the data in table 9 its effect gradually disappeared from the control soil to which no lime had been added. Both the cellulose decomposing bacteria and Azotobacter were too few in numbers to be significant in the results of this experiment.

It was expected that the addition of lime would decrease the acidity of the peat. The results of the pH determinations at the beginning and at the end of the experiment are recorded in table 9. For some obscure reason the pH of the control peat at the end of the experiment had increased nearly as much as that of the limed soils. Fortunately some of the original pulverized, moistened peat was placed in bottles which were stoppered with cotton plugs and stored in a separate room. The data show that the pH of this soil also had increased and was similar to that of the limed soils. Separate colorimetric pH determinations gave identical results. The decomposition of organic acids, which may take place in organic soils through good aeration, can hardly account for this great change in reaction. Since no logical reason can be offered for this phenomenon, the effects of lime on the microbiological activities in this soil cannot be attributed to changes in soil reaction.

DISCUSSION

The function of microörganisms in the transformation of organic matter in soil is a process of decomposition and synthesis. The rate of transformation depends upon many factors, chiefly moisture, temperature, aeration, food supply, and kind and number of microörganisms. The data presented in this paper show that under the conditions of the experiments food supply was of greater importance than were microörganisms. The microflora of the peat seemed to be adequate in numbers and in kind, because the introduction of the microflora of stable manure or of Palouse silt loam into the peat soil had no perceptible effect upon the number and kind of its microörganisms or their activities. Conversely, the food supply in the manure, consisting of readily available carbon and nitrogen compounds, greatly stimulated both numbers and activity of the microörganisms in the peat.

Bedford (2) mentioned the fact that the numbers of microörganisms of peat and mineral soils are wholly divergent when calculated upon the organic matter content of the soil. It has been pointed out in this paper that the number and activity of microörganisms are dependent upon the quantity of readily available carbonaceous and nitrogenous compounds in the organic matter as well as upon its total amount. Much of the organic matter in peat is extremely resistant to decomposition. The percentage of its total organic matter undergoing rapid transformation is so small that it is hardly comparable with that which is undergoing rapid transformation in mineral soils, especially in those cultivated mineral soils that are annually supplied with fresh crop residues or manure. The microflora of peat may differ from that of mineral soils in many respects independent of the total organic matter of the soil. A comparison of the microbiological activities of the woody-sedge peat to which wheat straw and sodium nitrate had been added with those of a fertile Palouse silt loam is graphically expressed in figures 2 and 3. The data for the Palouse silt loam were obtained in this laboratory in a separate experiment to be reported in a subsequent paper. This mineral soil contained 3.1 per cent organic matter, 0.145 per cent nitrogen, and only a trace of nitrate nitrogen when the experiment was started. Its reaction was pH 6.9. As may be seen from table 1, the woody-sedge peat contained over 60 per cent organic matter, 1.93 per cent nitrogen, 21 p.p.m. NO_3, and its reaction was pH 4.3. Both soils received approximately the same percentage of wheat straw and sodium nitrate. The differences in microbiological activities between the two soils are obvious. The sum of these activities measured in CO_2 evolution was greater in the peat soil than in the mineral soil. The reverse was true for the amounts of nitrogen liberated as NO_3. The numbers of bacteria and actinomyces were much larger in the mineral than in the peat soil but the opposite was true for the numbers of fungi. The sum of the average number of bacteria, fungi, actinomyces, and

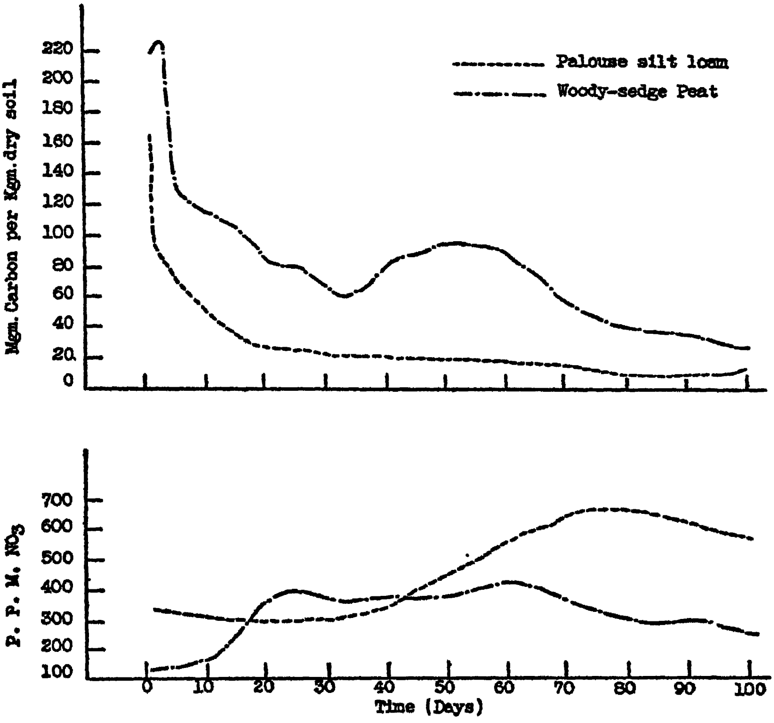

FIG. 2. CARBON DIOXIDE EVOLUTION AND NITRIFICATION IN PALOUSE SILT LOAM AND WOODY-SEDGE PEAT TREATED WITH WHEAT STRAW AND SODIUM NITRATE

cellulose decomposing bacteria per gram of dry soil was nearly 800 million in the mineral soil, whereas it was less than 500 million in the peat. The smaller of microörganisms in the peat liberated the greater amount

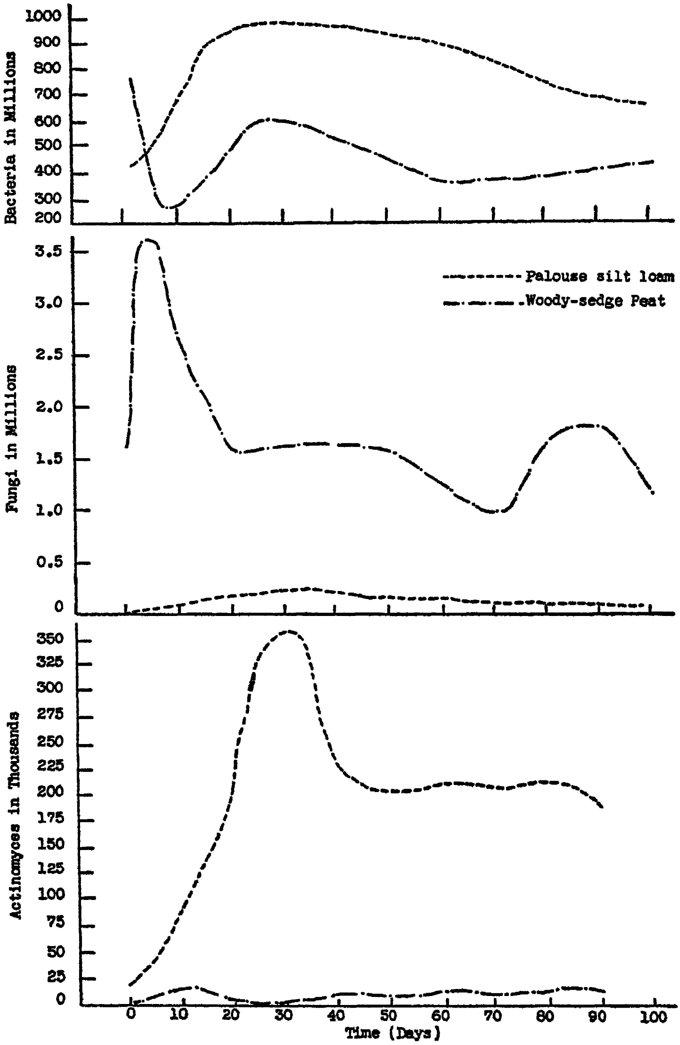

FIG. 3. BACTERIA, FUNGI, AND ACTINOMYCES IN PALOUSE SILT LOAM AND WOODY-SEDGE PEAT TREATED WITH WHEAT STRAW AND SODIUM NITRATE

The ratio of carbon liberated as CO_2 to the nitrogen accumulated as NO_3 and NH_3 was 37:1 for peat and 7:1 for Palouse silt loam. Marked differences occurred in the amounts of carbon liberated and in the numbers of bacteria, fungi, and actinomyces. These are variations existing between a mineral and a peat soil, both receiving similar treatments and kept under identical conditions of aeration, moisture, and temperature. It is evident that the amount of organic matter, regardless of its resistance to decomposition or its composition, is inadequate as a means for computation of microbial activities in soil.

It has often been stated in the literature that a large part of the decomposition of organic matter in peat is a result of the action of fungi. Since fungi were much more numerous in the peat than in the mineral soil referred to it might be supposed, and it frequently is, that a large part of the carbon liberated in peat in excess of that in the mineral soil, as shown in figure 2, may be accounted for by the action of fungi. It must be remembered that the addition of lime to the peat caused a threefold increase in CO_2 production and simultaneously a 50 per cent decrease in numbers of fungi. This indicates that the comparatively large amount of CO_2 liberated from the peat cannot be explained on the basis of fungous activity.

Although the addition of lime stimulated CO_2 evolution it did not cause any increase in the sum of the average numbers comprising the various groups of microörganisms as determined in these experiments. Waskman and Stevens (17) and Shunk (9) observed a similar increase in CO_2 production following the addition of lime to peat, but Shunk found a simultaneous increase in the number of bacteria. It seems that the most important effect of the lime added to the woody-sedge peat was that of bringing about certain changes which caused an increase in those carbonaceous compounds that are readily available for decomposition by the soil microflora. Although the total number of microorganisms was not altered materially the relationship between groups of microorganisms was changed significantly. This relationship changed in the direction of that existing in the mineral soil, which presumably contained a large percentage of readily available carbonaceous compounds. Since both soils were well supplied with available nitrogen, it seems reasonable to suppose that the change in relationship in numbers between groups of microörganisms brought about by the addition of lime to peat was due primarily to an increase in readily available carbon compounds.

SUMMARY

A virgin woody-sedge peat was treated with unsterilized stable manure, sterilized stable manure, wheat straw, superphosphate, sodium nitrate, and lime, to study the effect of these substances on the numbers of various groups of microörganisms and their activities. The numbers of microörganisms were determined at regular intervals by means of direct microscopic counts and by counts of colonies developing on plates prepared with selective culture media. Their activities were ascertained periodically by determinations of carbon evolved as CO_2 and of nitrogen accumulated as NO_3 and NH_3. The addition of stable manure, or wheat straw to which sodium nitrate had been added to equal the nitrogen content of the manure, increased CO_2 evolution and also the numbers in the various groups of microörganisms, except those of the cellu-

lose decomposing bacteria and the Azotobacter. It also increased the accumulation of nitrates but not of ammonia.

The microflora of unsterilized stable manure had no perceptible effect opon the numbers in the various groups of microörganisms in the soil or upon their activities. It was indicated that the beneficial effect of stable manure on the microbiological activities of the woody-sedge peat was derived from the readily available carbon and nitrogen compounds in the organic matter of the manure. Stable manure and wheat straw gave comparable results.

The addition of superphosphate or sodium nitrate caused no material increase in the total number of microörganisms, or in numbers in the various groups of microörganisms, and had no marked effect upon their activities in the soil.

The addition of lime greatly stimulated the liberation of CO_2 but did not cause any marked increase in total numbers comprising the various groups of microörganisms. It seemed to benefit Azotobacter to the extent that their numbers remained constant in the soil. It was apparent also that it caused an increase in the percentage of readily available carbon compounds in the organic matter of the peat as shown by a change in relationship in numbers between groups of microörganisms in the direction of that existing in a fertile mineral soil.

The total numbers of microörganisms and the relationship of various groups of microörganisms in peat and in a mineral soil similarly treated are compared. It is indicated that the total number of microörganisms, the numbers in various groups, and their activities in peat and in mineral soil may vary in many respects.

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THE LAWS OF SOIL COLLOIDAL BEHAVIOR: VIII. FORMS AND FUNCTIONS OF WATER[1]

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Water is associated in various forms with the colloidal materials of soils:

COMBINED WATER

First we have the constitutionally combined water of the hydrous oxides of aluminum, iron, and silicon and their hydrous compounds. This water can be driven off only by strong ignition. The best available data showing the relation of this water to the composition of soil colloids are found in the work of Robinson and Holmes (18). It is believed that a further systematic study in this direction would yield important information in regard to the constitution of the colloidal material, to its degree of hydrolysis, and to the relation of the latter to ion adsorption and exchange. The previously discussed isoelectric precipitates are therefore now being studied in respect to their combined water.

HYGROSCOPIC WATER

Next we have the surface attracted water or the hygroscopic water which is held by a powerful molecular attraction. This innermost film of water is subjected to a considerable compression, which has been estimated in the case of charcoal to a force equivalent to over 20,000 atmospheres (8). We may assume the heat of wetting to be a result of this compression. The compression is evident from the smaller apparent specific volume of colloids in water than in liquids like toluol and petroleum ether (16) in which the molecular attraction and the resulting compression are weaker.

The following figures for two of the soil colloids here studied are of interest. The figures are from the work of Anderson (1) and of Anderson and Mattson (2).

SOIL COLLOID	$\frac{SiO_2}{R_2O_3}$	HEAT OF WETTING PER GRAM	H_2O ADSORBED OVER 30 PERCENT H_2SO_4 PER GRAM COLLOID	SPECIFIC VOLUME IN WATER	SPECIFIC VOLUME IN TOLUOL	DIFFERENCE
		calories	*gm.*			
Sharkey	3.11	16.3	0.1605	0.368	0.387	0.019
Norfolk	1.60	7.6	0.0845	0.369	0.379	0.010

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[1] Journal Series paper of the New Jersey Agricultural Experiment Station, department of soil chemistry and bacteriology.

It is seen that the heat of wetting parallels the hygroscopicity. The differences in the specific volume in the two liquids indicate the volume contraction of the adsorbed water or rather the excess in the volume contraction of the water over that of the toluol. The figures are somewhat lower than the corresponding values for starch as given by Rodewald (19).

That the force of molecular attraction decreases rapidly as the distance increases is evident in various ways. Thus the surface tension of a film should show a decrease when the thickness of the film becomes less than twice the range of the molecular attraction. It was found by Rücker and Reinold (20) that for water there was no decrease in surface tension before the thickness of the film was reduced to $10\mu\mu$ ($= 1 \times 10^{-6}$ cm.). Again the vapor pressure of water droplets does not show an appreciable increase until their diameter falls considerably below 1μ. This indicates that the surface of a droplet of this dimension is to the molecule like a plane surface, since within the range of molecular attraction there is no effective curvature. Edser (5) states that the force of molecular attraction varies inversely as the 8th power of the distance.

The thing of interest in connection with our present subject is the quantity of water which is thus attracted to the surface of the colloid with the evolution of heat. The drier the colloid the greater is the quantity of heat liberated upon wetting. At what moisture content does the colloid cease to give off heat when wetted? The work of Rodewald (19) on starch showed the heat of wetting of this substance to be zero at a moisture content of 31.63 per cent, and Mitscherlich (15) found the heat of wetting of peat and of a heavy clay to approach zero at a moisture content of about 20 gm. of water in 100 gm. of dry soil. This moisture content is equivalent to the hygroscopicity of the materials. In soil colloids this varies with the composition and is in general higher the higher the ratio of silica to sesquioxides (2). The quantity of water thus attracted by molecular forces to the surface of colloids is therefore not very great, not nearly as great as the quantity of water that may, under certain conditions, be osmotically imbibed by the colloid.

What then is the order of magnitude of this, the hygroscopic film of water which is so tenaceously held by the particles? If we assume a uniform particle size we can calculate the ratio $r:r_1$ where r is the radius of the solid particle and r_1 the radius of the particle plus its layer of hygroscopic moisture. One gram of dry soil colloid, sp. gr. 2.65 occupies $\frac{1}{2.65}$ cc. = 0.378 cc. This would make a sphere with a radius

$$r = \sqrt[3]{\frac{0.378}{4/3\ \pi}} = 0.448 \text{ cm.}$$

If the hygroscopic moisture is 0.2 cc. per gram dry soil colloid, e.g., the approximate value for the Sharkey soil colloid, then our 0.378 cc. solid sphere would be covered with this water and we would have a 0.578 cc. sphere with a radius $r_1 = 0.518$ cm. In 1 gm. of soil colloid there are billions of particles but if they were all spheres of uniform size the ratio $r:r_1$ would be the same, or 448:518. If, therefore, we knew the particle size we would know the approximate thickness of the film.

By ultramicroscopic count the Sharkey soil colloid, dispersed by ammonia, yielded an average particle radius, of $45\mu\mu$ (2). If this were the ultimate size then the average thickness of the film would be about $52 - 45 = 7\mu\mu$. But by electrodialysis, by saturation with NaOH, and by diffusion through a Pasteur-Chamberland filter the author later estimated, by comparing the volume of floc of the Na-saturated Sharkey with the volume of floc of the ammonia treated colloid (11), a particle radius of $26\mu\mu$. (The Na-saturated particles were not all visible in the ultramicroscope.) If we take this as the ultimate size of the particles then we find by the foregoing ratio a thickness of the film equal to about $4\mu\mu$.

Although this estimate of the thickness of the hygroscopic moisture film is based on several assumptions it is significant that the order of magnitude is very nearly the same as that arrived at by P. Ehrenberg (6). Basing his conclusions upon theoretical considerations as well as upon a rich array of experimental data this authority fixes the thickness of the hygroscopic moisture film at $2.5\mu\mu$ or equal to about 10 molecules of water. Mitscherlich accepts the thickness of the film as given by Ehrenberg and uses it as a basis for calculating the total surface of a soil (16).

The thickness of the film of molecularly attracted water $(r_1 - r)$ must therefore be considerably less than the radius of the particles. In the Na-saturated condition certain soil colloids swell and imbibe so much water that, on the same basis of calculation, the thickness of the water envelope is nearly twice the radius. That this very great but less powerful inbibition of water is due to osmotic forces will be shown later.

The expansion or swelling of a porous material resulting from the intake of the molecularly attracted water is manifested by great energy. Thus Rodewald (19) found that it required 2073 kgm. per cc. to prevent dry starch from swelling in the presence of water. Because of the loose structure of soils the swelling resulting from the intake of the molecularly attracted water is not so marked. The great force of expansion of dry wood when wetted is a familiar example of the energy of this form of swelling.

As to the nature of this innermost film of molecularly attracted water we have no definite knowledge. The molecules of water in this film occupy probably an oriented position as distinguished from the random distribution in the interior of the mass of water. The water adsorbed by molecular attraction must therefore possess different properties as compared to free water. It is more dense and must have a different viscosity or, indeed, be quite rigid like a solid, especially in the innermost layers. It does not freeze even at -70°C., as shown by the work of Bouyoucos (4). The dielectric constant is probably also different. The electrical polarity in this oriented layer of water molecules, if known, would probably explain why air bubbles and all inert substances, which do not dissociate diffusible ions, become negative in water. By virtue of this polarity the distribution of the OH and H ions of the water may be such that the former will predominate within the adsorption film and thus impart a negative charge, not to the particles (or the air in the air bubbles) but to their surrounding film of adsorbed water whereas an excess of H ions charges the outside liquid to positive. The dissociation constant of water may even be different from that of free water, in which case the process of hydrolysis may be greatly modified.

CAPILLARY WATER

Apart from any molecular attraction or osmotic force (see the following) a certain quantity of water will be held in the fine capillaries of the soil or colloid by virtue of the surface tension. Since the water wets the soil with great energy and thereby covers each particle with a thin film and since the surface tension tends to reduce the surface to a minimum it is obvious that water will be pulled up against gravity and fill the finest capillary spaces.

The height h to which water will rise in a circular capillary may be calculated from the familiar equation:

$$\pi r^2 h \; g = T \; 2\pi r$$

where T is the surface tension in dynes per centimeter hence

$$h = \frac{2\,T}{r\,g} \text{ cm.}$$

The height to which water will rise against gravity varies therefore inversely as the size of the capillaries. The force with which water is held in the most minute capillaries is quite enormous. Thus Lebedeff (9) found that water could be removed from all the soils which he studied up to a centrifugal force equal to 18,000 times the force of gravity. Between 18,000 and 70,000 × gravity the moisture content remained almost constant and represented the molecularly attracted, or hygroscopic water, being about 6 per cent in the Sassafras silt loam and about 31 per cent in a clay soil. The last water to be removed by the centrifuge is probably somewhat subjected to the force of molecular attraction and is therefore not entirely free capillary water held by the surface tension alone.

The free capillary water which is held or lifted up in the capillaries solely by the force of surface tension must exert a contracting and a downward pull tending thereby to reduce the distances between the particles and oppose any tendency to swell. The function of the capillary water is therefore opposite to that of both the hygroscopic water and the osmotically held water, which cause the absorbent to swell. The energy of swelling due to the molecularly attracted water is so great that any opposition offered by the downward pull of the capillary water becomes insignificant. The energy of swelling due to osmotic imbibition is rather small. The opposing force of the downward capillary pull is here more equally matched and keeps the osmotic swelling in check. Thus if a Na-saturated soil colloid in the process of imbibition is required to lift the water against gravity, as when placed in a funnel provided with a stem several feet long, the swelling is greatly reduced.

The study of the capillary forces in dispersed materials will show no definite relationship between the various factors unless a completely inert powder is used. In the case of reactive materials like soils which dissociate diffusible ions we shall encounter an osmotic imbibition. These osmotic forces will extend across the minutest capillaries and part way across the larger ones. The force by which the water is held or the height to which it is lifted is therefore by no means an expression of the capillary forces alone. Under certain conditions the capillary forces are altogether eliminated. The Na-saturated soil colloids not only completely fill all capillary spaces, by osmotic imbibition, thus destroying every meniscus, but their particles are pushed a considerable distance apart. The particles become suspended, so to speak, in the osmotically imbibed liquid, in cushions of their "micellar solutions." We shall now turn our attention to this form of water.

THE WATER OF OSMOTIC IMBIBITION

The various forms and functions of soil water discussed in the foregoing pages have long been more or less definitely defined and have been the subject of an extensive study by many investigators. This cannot be said about the water of osmotic imbibition, which forms the main object of our present study. Where this form of water has been recognized it is because it could not have escaped recognition, producing as it does in many instances the most conspicuous effects. The forces with which it is associated in the soil have never been accounted for nor have its functions ever been formulated. This may not have been possible without the conception of the soil colloidal micelle as presented in the preceding articles (12).

Soil colloids saturated with alkali cations have, because of their great swelling, been classed as lyophillic colloids as distinguished from the lyophobic behavior of the same materials when saturated with divalent cations (7). But no satisfactory explanation of this influence of the nature of the exchangeable ions has been given. It has been suggested that the imbibed water represents the water of hydration of the ions. But apart from the fact that the imbibed water is often much too great to be thus accounted for there are other reasons which make this explanation unacceptable. These will be brought out later.

The enormous swelling exhibited by certain samples of bentonite has been ascribed to a micaceous structure, a "one-dimensional" colloid. The bentonites and many of the soil colloids apparently contain plate-like crystals but it is difficult to see why this should be a necessary condition for swelling and other colloidal properties. As far as the surface is the controlling factor we should expect a greater effect if the particles were reduced to colloidal dimensions in two directions, that is, if the platelets were cut into narrow bars, whereas the greatest effect would be produced by a "tri-dimensional" colloid, that is, by cutting the bars into cubes.

In the first of this series of articles it was explained how we may account for the swelling as an osmotic phenomenon (12). If the colloidal particles dissociate diffusible ions they will be surrounded by a swarm or atmosphere of these ions. The electrostatic attraction will prevent these ions from diffusing very far away from the surface of the particle which, through the dissociation, has become a highly multivalent ion complex of opposite charge. This means that the osmotic pressure will be higher near the surface of a particle than it is in the outside medium. The ion density in the micellar atmosphere appears to decrease rapidly with the distance from the surface. The osmotic pressure and the force with which water is imbibed are therefore much greater the lower is the water content of the gel. We shall show later that a definite relationship exists between the pressure and the water content.

A highly dissociated cation such as the Na ion must cause a greater swelling of a negative colloid than when the latter is saturated with a less dissociating ion like the Ca ion. This is well known to be the case. Further, the addition of electrolytes must, according to the Donnan equilibrium, suppress the swelling of the colloid. This suppression should be governed by the concentration and by the valence of the ions of the free electrolyte in the solution. This relationship was experimentally verified in the work referred to.

To avoid too much repetition we shall confine ourselves to the following diagrammatic presentation which the author has found useful in making clear the application of this theory to the colloidal micelle:

In figure 16 are shown two spheres representing a Na-saturated and a Ca-saturated micelle. The micelle consists of the colloidal particle or ion complex; the atmosphere of dissociated, diffusible ions; and the osmotically imbibed water. The relation between the size of the particles and the thickness of the layer of osmotically imbibed water, as shown in the figure, was calculated from the volume occupied by 1 gm. of the Na- and Ca-saturated Sharkey soil colloid after complete inbibition of water. The respective volumes were 7.25 and 1.86 cc. If we assume a specific gravity of 2.65 and that the particles are spherical and all of the same size, we get the relationship shown in the figure.

The very great imbibition of water by the Na-saturated colloid as compared to that of the Ca-saturated material is to be ascribed to a greater dissociation of the Na ions. This causes a greater osmotic pressure around the particles, which are therefore pushed further apart by the greater osmotic inflow of water.

The swelling of the soil colloid saturated with any cation will be at a maximum in the absence of free electrolytes. To illustrate how the free electrolyte causes a suppression of the swelling we shall choose a specific case and confine ourselves to the simplest effect, that of a uni-univalent salt.

Let v_1, figure 16, represent a small volume within the micellar solution of a

Na-saturated colloid and v_o an equal volume in the outside solution. Assume then that the concentration z of the micellar ions, i.e., ions dissociated by the particles, is such that there is an average of 12 ions in v_i. If there were no free ions in the dispersion medium (an ideal case never realized) then the osmotic pressure would be directly proportional to z and would be at a maximum.

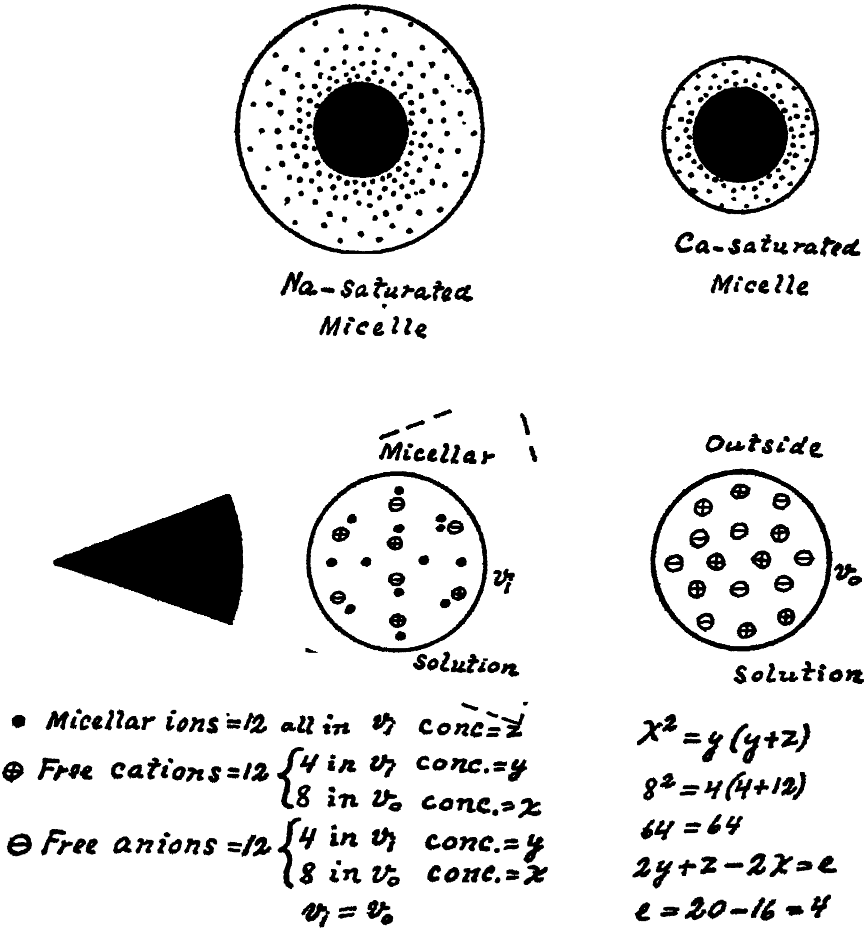
Na-Saturated Micelle
Ca-Saturated Micelle
Micellar
Outside
v_i
v_o
Solution
Solution
● Micellar ions = 12 all in v_i conc. = z
⊕ Free cations = 12 {4 in v_i conc. = y; 8 in v_o conc. = x}
⊖ Free anions = 12 {4 in v_i conc. = y; 8 in v_o conc. = x}
$v_i = v_o$
$x^2 = y(y+z)$
$8^2 = 4(4+12)$
$64 = 64$
$2y+z-2x = e$
$e = 20-16 = 4$

FIG. 16. THE DONNAN DISTRIBUTION OF IONS BETWEEN THE MICELLAR AND THE OUTSIDE SOLUTIONS

Now let the total outside solution be equal to the total micellar solution so that v_o and v_i represent the same fraction of each and suppose that we add so much of a uni-univalent salt, e.g., NaCl, to the system that if equally distributed there would be 6 cations and 6 anions in v_o and an equal number in v_i, or together 12 ions of each kind in v_o and v_i. What will be the ultimate distribution of the ions of the added salt at equilibrium and how will this distribution affect the difference in osmotic pressure between v_i and v_o?

According to the principle of a constant ion product the distribution must follow the Donnan equation, which, in application to the case here studied, demands that the product of the ions in v_i must equal the product of the ions in v_o thus

$$x^2 = y\,(y + z)$$

where x is the concentration of each of the ions of the salt in v_o and y is the concentration of these ions in v_i whereas z is the concentration of the Na ions dissociated by the complex.

To satisfy this equation the figure shows that at equilibrium there would be 4 cations and 4 anions of the added salt in v_i and 8 of each of these ions in v_o.

In regard to the second question we note that by summing up all the ions in v_i and all the ions in v_o we find an excess $e = 4$ ions in v_i whereas in the absence of any free electrolyte the excess ion concentration in v_i was equal to 12, i.e., since x and y are then both equal to zero, $e = z$. The greater we make x and y the smaller will be e as compared to z, and since the excess osmotic pressure in v_i is proportional to e it follows that the osmotic swelling of the colloid will be suppressed by the addition of a salt and that the greater the concentration of the salt the greater will be the suppression.

TABLE 87

Swelling of bentonite and the Sharkey soil colloid

Volume of gel in cubic centimeter per gram

TREATMENT	UNTREATED	ELECTRODIALYZED	Na-SATURATED	K-SATURATED	Ca-SATURATED
Bentonite	7.8	2.6	8.0	4.6	...
Sharkey	1.85	1.75	7.2	...	1.9

We shall now study the imbibition of water by soil colloids and see if we can harmonize the foregoing theory with our observations.

THE OSMOTIC SWELLING AS GOVERNED BY THE NATURE OF THE EXCHANGEABLE CATIONS

That the swelling of bentonite and of soil colloids depends upon the nature and quantity of the exchangeable cations present rather than upon any particular structure was shown by the author some years ago (11). The swelling of the colloidal materials was studied as follows: Burettes were cut into lengths of 5-cc. capacities and the ends ground even. These tubes were placed in an upright position on a few pieces of filter paper on glass slides and held in that position by means of rubber bands. Half a gram of the material which had passed through a 50-mesh sieve was placed in each of the tubes and the latter were then placed in a shallow dish containing sufficient distilled water to moisten the paper on which the material was resting. Water was imbibed through capillary rise and the volume occupied by the thoroughly wetted material was recorded as the swelling. The swelling of variously treated bentonite and the Sharkey soil colloid is shown in table 87.

By electrodialysis the bentonite lost its great swelling power. When the electrodialyzed bentonite was saturated with NaOH its power to swell was restored. In the K-saturated condition the swelling was intermediate between that of the Na-saturated and the H-saturated (electrodialyzed). The original bentonite contained no exchangeable divalent cations. The swelling of the bentonite is shown in figure 17.

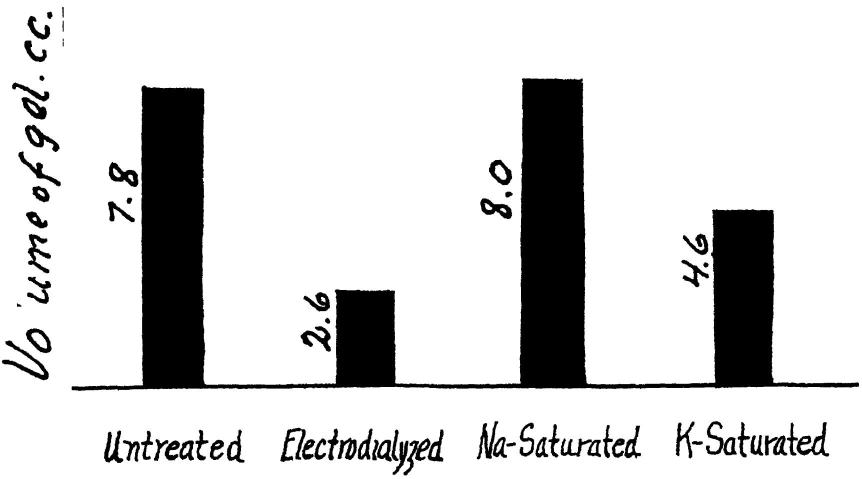

FIG. 17. THE SWELLING OF BENTONITE IN RELATION TO THE NATURE OF THE EXCHANGEABLE CATIONS

It was then found that if the Sharkey soil colloid was electrodialyzed and saturated with NaOH it developed a swelling power almost equal to that of bentonite.

The difference in the influence of Na and Ca upon the swelling of the Sharkey soil colloid is strikingly illustrated in plate 1. The electrodialyzed colloid was saturated with NaOH and $Ca(OH)_2$ in various proportion, a total of 0.80 m.e. per gram being taken. This quantity represents the exchange capacity of the colloid. Half-gram portions of the prepared samples were placed in a series of nine cylinders and allowed to imbibe water as described. The relative proportions of the two cations is shown in the figure where 100 represents full saturation.

The volume occupied by 1 gm. colloid after complete imbibition increased from 1.86 cc. of the Ca-saturated colloid to 7.10 cc. at full Na-saturation. A marked increase in swelling seems to take place after 20 per cent of the exchangeable Ca has been displaced by Na. This increase in swelling was accompanied by an increase in dispersibility and in the negative charge of the particles as shown in a previous publication (11).

OSMOTIC SWELLING AND THE CATION EXCHANGE CAPACITY

A very fundamental relationship was then observed. When NaOH was added in small increments to various electrodialyzed soil colloids it was found that the swelling increased to a certain maximum and then again decreased. In all cases the swelling was at a maximum when the quantity of NaOH added was equal to the exchange capacity of the colloid. An excess of free electrolyte suppressed the swelling. It was further observed that the maximum swelling the colloid attained depended upon the cation exchange capacity. The higher this capacity the greater was the swelling and vice versa. Lateritic soil colloids possessing a very low cation exchange capacity show practically no increase in swelling in the Na-saturated condition. (The capillary spaces be-

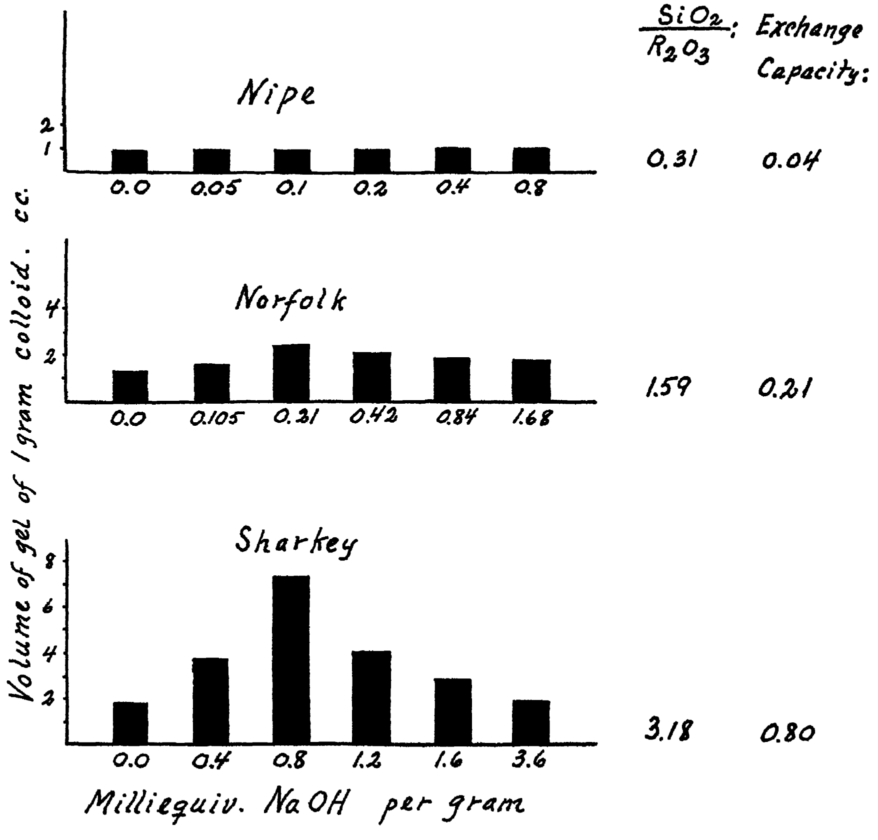

FIG. 18. THE RELATION BETWEEN SWELLING, COMPOSITION, AND EXCHANGE CAPACITY

tween the particles are evidently sufficient for the slight osmotic imbibition in these materials.) In figure 18 are shown the volumes occupied by the gel of 1 gm. colloid (110°C. basis) to which various quantities of NaOH have been added. The figure includes three soil colloids representing low, intermediate, and high cation exchange capacities.

Although the figure shows the differences most clearly, table 88 will be useful in showing (*a*) the volumes occupied per gram by the powdered (50-mesh), air-dry, electrodialyzed colloids after gentle tamping; (*b*) the volumes occupied by the gels per gram colloid in the electrodialyzed condition; and (*c*) the volumes of the gels per gram colloid when saturated with NaOH to the extent of their exchange capacities.

The experiment shows an unmistakable relationship between the power of soil colloids to adsorb and exchange cations and their power to imbibe water. The Nipe colloid with the very low exchange capacity of 0.04 m.e. per gram, showed practically no tendency to swell. Undoubtedly some water is osmotically imbibed even by this colloid but where the imbibition is very small it will be accommodated by the spaces between the particles in the porous mass of the material.

TABLE 88

The relation between exchange capacity and swelling

COLLOID	EXCHANGE CAPACITY	VOLUME PER GRAM AIR-DRY COLLOID	VOLUME OF GEL PER GRAM ELECTRODIALYZED COLLOID	VOLUME OF GEL PER GRAM Na-SATURATED COLLOID
	m.e.	*cc.*	*cc.*	*cc.*
Nipe	0.04	0.90	0.92	0.92
Norfolk	0.21	1.04	1.42	2.30
Sharkey	0.80	1.12	1.75	7.20

THE SUPPRESSION OF THE SWELLING BY ELECTROLYTES

It was shown in section I of this series (12) that, according to the osmotic equation deduced from the Donnan equilibrium, the swelling must be suppressed by the free electrolyte and that this suppression is governed wholly by the concentration and by the valence rule as explained. The swelling will also be suppressed, independently of any free electrolyte, whenever a highly dissociating cation (in a negative colloid) is displaced by a slightly dissociating cation. In this relation the Hofmestier series (which is governed by the specific nature of each ion and not alone by the valence) becomes manifest. Thus the swelling will be suppressed when Na is displaced by K and K by Ca.

We have seen that the Al and Fe ions will displace the exchangeable cations and that the complex thus formed has suffered a decrease in exchange capacity. The Al and Fe ions are not again displaceable by the neutral salt treatment

(13). This shows that these ions undergo very slight dissociation by the complex. Their introduction into the complex amounts to an artificial lateriza-

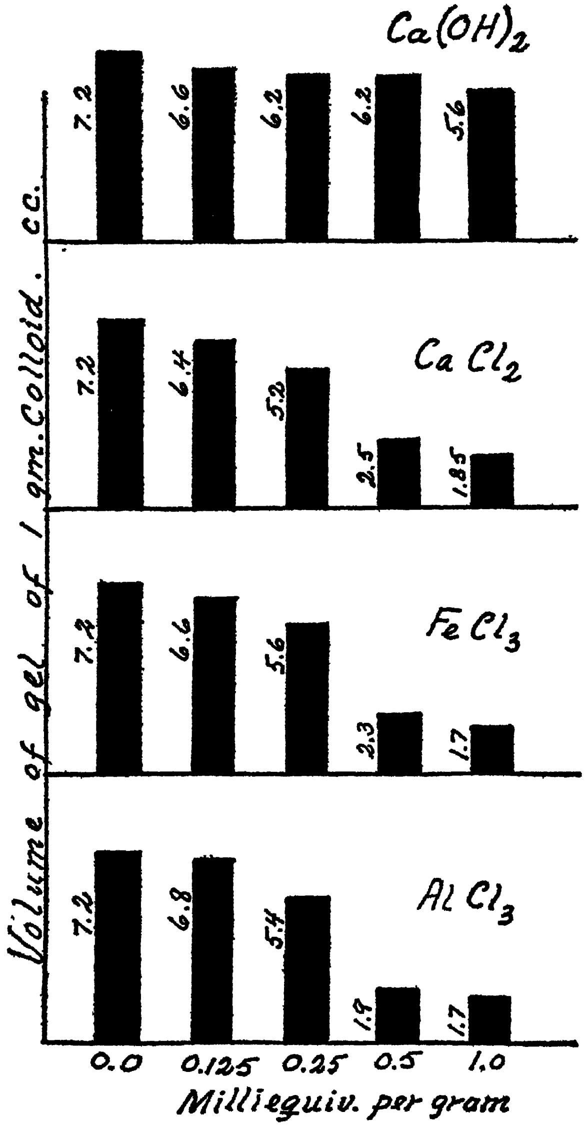

Fig. 19. The Suppression of the Swelling of Na-saturated Sharkey Colloid by Various Electrolytes

tion of the colloid. (Their linkage to the silicate valences may, however, be unlocked by hydrolysis in an alkaline medium.) We would therefore expect the greatest suppression of the swelling as a result of displacing the Na ions by Al and Fe ions. The Al ions should be the most efficient ones because we have seen that this ion causes the greatest decrease in the exchange capacity, the Fe combination being too readily hydrolyzed (13).

Figure 19 shows the suppression of the swelling of the Na-saturated Sharkey colloid by the addition of $Ca(OH)_2$, $CaCl_2$, $FeCl_3$, and $AlCl_3$ at the rate of 0.125, 0.25, 0.05, and 1.0 m.e. per gram colloid. Comparing the effect of the three chlorides we note that $CaCl_2$ causes a slightly greater suppression in the two lowest concentrations. If these differences are real they may be accounted for by the practically complete hydroxidation of the first small quantities of $AlCl_3$ and $FeCl_3$ by the alkali-saturated colloid. Where 0.5 m.e. of the chlorides is added we find the following suppression of the swelling

$$Al > Fe > Ca$$

Where 1.0 m.e. is added per gram colloid the swelling is suppressed to 1.7 cc. by Al and Fe and to 1.85 cc. by Ca. This represents, however, no exact measure of the true osmotic imbibition because the colloid plus the capillary water will occupy a certain irreducible volume. The osmotic imbibition may therefore be less than indicated by the smallest volume of the gels.

It is important to note that $Ca(OH)_2$, in the proportions used, caused a far smaller suppression of the swelling than do the chlorides. The $Ca(OH)_2$-treated colloid contains excess base and has an alkaline reaction. But after the $Ca(OH)_2$ was added to the Na-saturated colloid the latter was exposed to the air during the drying. The hydroxides, $Ca(OH)_2$ and NaOH (the latter formed by displacement), must therefore be largely converted into carbonates. The $CaCO_3$, being only slightly soluble, does not appreciably suppress the swelling as a free electrolyte, and because of the low Ca ion concentration in the solution there will be only a moderate displacement of the Na ions in the complex. The result is that the complex remains highly saturated with sodium and retains therefore to a great extent its capacity to swell. This emphasizes the fact long recognized that lime is not the proper agent to be used for improving the condition of alkali soils.

RELATION BETWEEN PRESSURE AND WATER CONTENT

If a suspension of a soil colloid is placed on a filter sufficiently fine to retain the particles, a certain amount of the water will be removed by gravity while another portion of the water will be retained by the colloid. The quantity of water thus retained will depend upon the cation exchange capacity of the colloid and upon the nature of the displaceable cations. The quantity of water retained by the colloid will also be affected by the presence of electrolytes and by the valence of the ions as demanded by the Donnan equation (12). A bentonite, which has an exchange capacity of about 1 m.e. per gram retains in the Na-saturated condition, nearly 9.5 cc. of water per gram solid material. At this water content the suspension has already assumed the consistency of a gel. This is remarkable in view of the fact that the solid phase (sp. gr. = 2.65) occupies only about one twenty-fifth of the volume of the gel. If spherical particles of uniform size are assumed their distance apart would be nearly four times their radius.

The water which is removed by gravity represents the free, outside or intermicellar solution whereas the water which is retained within gel constitutes the micellar solution. The latter is more or less rigidly held by the osmotic force created by the micellar ion atmosphere. When the free, intermicellar solution is removed the micelles come into intimate contact and are no longer free to move about. In place of a viscous fluid there results, therefore, a more or less rigid gel.

TABLE 89

Quantities of water, in cubic centimeter per gram oven-dry (110°C.) material, retained by bentonite gel against different pressures

PRESSURE, P	WATER, w	$w \cdot \sqrt[3]{P}$	$\frac{r_1 - r}{r}$	$\left(\frac{r_1 - r}{r}\right) \cdot \sqrt[6]{P}$
cm. Hg	*cc.*			
0.2	9.36	5.52	1.97	1.52
1.2	7.16	7.62	1.72	1.77
5.6	5.93	10.55	1.56	2.09
10.5	4.94	10.82	1.42	2.10
13.8	4.38	10.51	1.34	2.08
20.5	3.95	10.81	1.26	2.08
30.0	3.37	10.49	1.16	2.05
41.0	3.14	10.86	1.11	2.06
50.0	2.96	10.95	1.07	2.06
61.0	2.71	10.70	1.02	2.03
70.0	2.57	10.62	0.99	2.01

To remove any part of the micellar solution, pressure must be applied and this pressure must be equal to the opposing osmotic pressure. In the first of this series of papers (12) it was shown that the ratio of $\frac{x}{y}$, that is, the Donnan distribution of the free electrolyte, increased rapidly with a decrease in the water content of the gel. This indicates a rapid increase in the micellar ion concentration (z) as we pass from the outermost to the innermost layers of the ion atmosphere. The pressures required to remove successive quantities of water from the gel should therefore increase in the same proportion and should represent the osmotic pressure at various "depths" in the micellar atmosphere.

In order to determine the relationship between the pressure and the water retained by the gel the following experiment was performed. A thick, yet fluid, suspension of bentonite containing 100 gm. solid matter in 1,500 cc. water was prepared. Portions of this suspension were placed in Büchner funnels with fused-in sintered filter discs of fine porocity. By means of a vacuum pump and a mercury manometer the pressure in the filter flasks was kept at the desired point by frequent adjustment until no more water was removed from the gel. The water was caught in a narrow graduated test tube placed inside the flasks. Evaporation was prevented by covering the funnel with a rubber stopper into which a glass tube, drawn at the upper end into a very fine capillary, was inserted. The water remaining in the gel at the various pressures was determined by drying at 110°C.

The results are shown in table 89, in which the first column gives the pressures in centimeters of mercury. The second column gives the number of

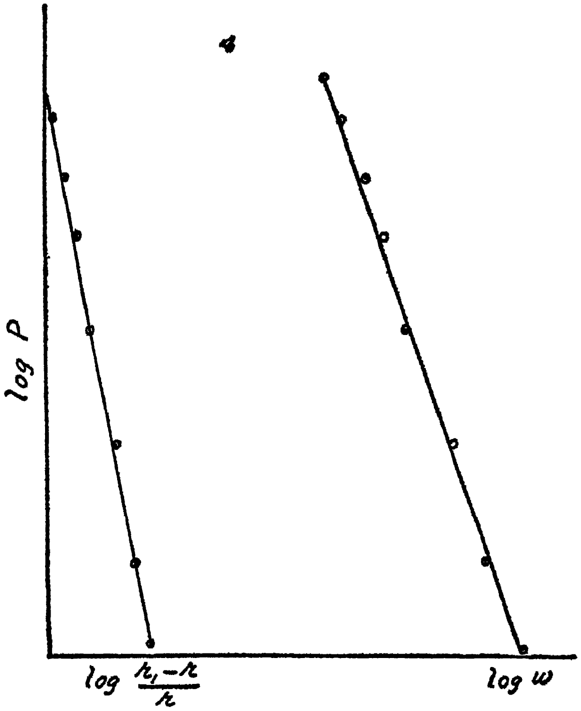

Fig. 20. The Relation Between the Volume of Water (w) per Gram Bentonite (Also Between the Thickness of the Water Envelope of the Particles, $\frac{r_1 - r}{r}$) and the Pressure (P) Applied

cc. of water retained by one gram oven-dry colloid. The third column gives the products of $w \cdot \sqrt[8]{P}$ where P = pressure and w = water as given in the preceding columns. The fourth column gives the thickness of the osmotically imbibed water envelope in terms of the radius r of the particles which are assumed to be spherical and of uniform size. These values are derived as follows: 1 gm. dry colloid, sp. gr. = 2.65, represents a solid sphere = 0.378 cc. with a radius r = 0.448 cm. The volume v of the gel is 0.378 + w cc.; r_1 is the radius of a sphere of volume v. The radius r_1 represents, therefore, the radius r

of the solid sphere plus the thickness of the water envelope which is $r_1 - r$. In terms of r as a unit of measure the thickness of the water envelope is $\frac{r_1 - r}{r}$. If now the individual particles were all spherical and of the same size this relationship would remain the same. Although this is only an approximation, it may be assumed that the ratio of the thickness of the water envelope at the various water contents to the "average effective radius" is very nearly proportional to the values in column four. The fifth column gives the product of the thickness of the water envelope and the sixth root of the pressure.

It will be noted that the pressure necessary to remove more water from the gel increases rapidly as the water content decreases. With the exception of the two lowest pressures the mathematical relationship is such that the cube root of the pressure multiplied by the water content is equal to a constant, the average value of which is 10.70. The increase in pressure is therefore inversely proportional to the cube of the water content. Since the volume of a sphere is proportional to the cube of the radius this would mean that the pressure increases inversely as the sixth power of the radius or as the sixth power of the distance from the solid surface. It will be seen that the $\frac{r_1 - r}{r}$ values when multiplied by the sixth root of the corresponding pressures yield approximately constant values, except, as before, in the case of the first two figures.

If $\log P$ is plotted against $\log w$ on the one hand and against $\log \frac{r_1 - r}{r}$ on the other, as in figure 20, straight lines with a slope of 3 and 5.6 are respectively obtained. It should be pointed out that the cubes of the values $r_1 - r$, which represent the thickness of the water envelopes, are only approximately proportional to the volume of the water, the deviation becoming greater the lower the water content. The slope of the corresponding line is therefore less than 6.

The range over which this water and pressure relationship holds has not been determined. Equipment for this study is now being procured and the results will be reported later.

INTERNAL PRESSURE

At high water content of the gel, the product $w \cdot \sqrt[3]{P}$ decreases rapidly; the gel does not imbibe as much water as is demanded by the relationship. To explain this let us briefly review the theory of the imbibition. It is assumed that the force which opposes the applied pressure is (above a certain moisture content where water exists outside the range of molecular attraction), an osmotic force created by the ions dissociated by the colloidal particle. These ions tend to diffuse in all directions but are held in check by the electrostatic attraction of the oppositely charged colloidal ion complex which forms the nucleus of the ion atmosphere. It is this attraction which limits the swelling or osmotic imbibition, which would otherwise take place until the osmotic pressure was the same throughout the system, that is, until the distribution of the ions was equalized. The electrostatic restraint upon the diffusion of the dissociated ions constitutes therefore an internal pressure which acts in opposition to the osmotic pressure. This internal pressure appears to equal about 1.5 cm. of mercury or about 0.02 of an atmosphere as determined by extrapolation of $w \cdot \sqrt[3]{P} = K$.

It will be recalled (12) that by applying the osmotic equation $2y + z - 2x = e$ to the distribution of ions between the inside and outside solution of a bentonite gel after complete imbibition of NaCl solutions the value of e was nearly constant in all cases and equal to a concentration of about 0.0008 molar (compare table 3). This corresponds to about 0.02 of an atmosphere, which is the same as here estimated from the water content-pressure relationship. There exists therefore at equilibrium a residual osmotic pressure which is unable to cause a further imbibition of water. If there were no opposing force the gel would swell until $e = 0$. But this would lead to an equal distribution of the micellar ions and to their separation from the colloidal ion complex. This is, however, prevented by the electrostatic attraction which reaches a critical maximum when the micellar ions have diffused out into the surrounding medium a certain average distance. An equilibrium is then established and the gel will swell no more. It is significant that this theoretical deduction is supported by two so different experimental methods which both yield the same approximate values in terms of osmotic pressure. In its electrical equivalent this internal pressure expresses itself in the potential difference of the double layer, that is, the electrokinetic potential.

DEPRESSION OF THE FREEZING POINT

Bouyoucos (3) found that the depression of the freezing point of soils increased in a geometric progression whereas the percentage of water decreased in an arithmetic progression. If we plot the logarithms of the depressions against the logarithms of the water content per gram soil as reported by Bouyoucos we get approximately a straight line with a slope not very different from that of the corresponding graph in figure 20. This is exactly what we should predict on the basis of the theory here presented. But Bouyoucos gives another interpretation. He does not recognize the existence of a micellar ion atmosphere but believes that the depression of the freezing point is entirely due to the soluble electrolytes of the soil. To explain the geometric instead of an arithmetic progression of the depression he assumes that a portion of the water held by soils is unfree or unavailable to act as a solvent. Thus if 15 per cent water is rendered inactive as a solvent by a clay, then at 40 per cent moisture content there would be 25 per cent available, and at 20 per cent moisture content only 5 per cent would be available to dissolve the salts. The soil solution would then be five times instead of twice as concentrated at the lower moisture content.

This view, which has been adopted by some investigators, is, in the author's opinion, unsatisfactory. If the molecularly attracted water cannot act as a solvent how then can we account for the almost instantaneous reaction of soils with ions as in base exchange reactions? Bouyoucos' assumption might be tested in several ways. If his explanation is correct there should be no increase in the depression if, as in our present experiments, the various moisture contents were arrived at by withdrawal of the solution by pressure instead of adding decreasing amounts of water to the dry soil as was done. Secondly an electrodialyzed soil free from diffusible electrolytes might be used. In either case our present theory would demand a geometric progression in the increase of the freezing point depression.

It is true that the hypothesis of Bouyoucos would account for the "negative adsorption" which we have here explained on the basis of the Donnan distribution. But it will not account for the valence effect nor for the effect of the concentration of the free electrolyte unless we make the very improbable assumption that the quantity of "unfree" water varies accordingly. The fact that the "negative adsorption," the swelling, the quantities of water retained against various pressures, the viscosity (and, it might be predicted, the depression of the freezing point) are directly related to the quantity and the nature of the diffusible ions in combination with the colloid indicates that all these phenomena, and many others, are *functions of a colloidal ionogen.* The hydration of the ions, on the basis of which some investigators would account for the lyophillic behavior of colloids, is here held to exert an indirect effect in that the most highly hydrated ions undergo the highest degree of dissociation by the complex. The effect of the concentration and valence of added electrolytes upon these functions can be satisfactorily accounted for only by the Donnan equilibrium.

THE COMPOSITION OF THE MICELLAR SOLUTION

We have seen that the micellar solution must be less concentrated with respect to free electrolytes than the outside or intermicellar solution. This enables us to make some very interesting predictions which have in part been verified and which are in part under investigation.

The first deduction is that if, by pressure, we remove the micellar liquid from a fully imbibed colloid, in equilibrium with the outside solution, we shall find the concentration (y) of the expressed solution lower than the concentration (x) of the (outside) solution which runs off under the influence of gravity alone. Further, if the micellar ion concentration (z) increases rapidly in the direction of the interface then the concentration of the ions of the free electrolyte will decrease in the same direction. The expressed solution should therefore become more and more dilute as we increase the pressure.

We may choose any ion and, if it be the H ion, we should find an increase in pH of the expressed liquid as the pressure is increased.[2] This will at first sight seem like a paradox, for we have long known that a moist *negative colloid* or its suspension reacts more acid than its extract. Figure 21 will, however, serve to explain the apparent contradiction. It represents a section (*a*) of a particle, (*b*) of the micellar solution, and (*c*) of the outside solution. The complex exists partly in combination with H ions which undergo a certain degree of dissociation, diffuse into the micellar solution, and establish a concentration gradient indicated by the size of the letter *z*. In the solution there are also a certain number of free H ions which are paired with diffusible anions. At equilibrium the equation

---

[2] This is true only as far as free acid is present. If the medium is alkaline the pH of the expressed liquid must decrease. The same distribution law holds of course for the H^+ and OH^- ions of the water as well as for any extraneous ions, but since $[H^+]\cdot[OH^-] = K_w$ the dissociation of the water will be affected by removal of the latter from the micellar solution.

$$x^2 = y\,(y + z)$$

must be satisfied no matter which part of the system we consider. Where *z* is larger *y* must be smaller. The *y* concentrations must therefore grow smaller, as indicated by the size of the letter *y* in the figure.

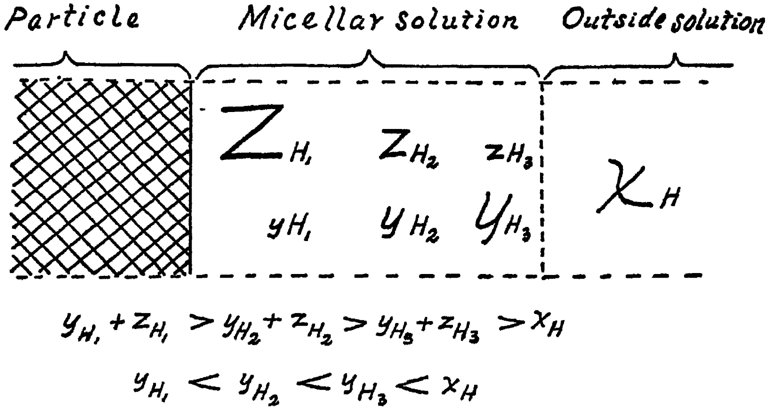
Particle
Micellar Solution
Outside solution
Z_{H_1}
Z_{H_2}
z_{H_3}
y_{H_1}
y_{H_2}
y_{H_3}
X_H
$y_{H_1} + z_{H_1} > y_{H_2} + z_{H_2} > y_{H_3} + z_{H_3} > x_H$
$y_{H_1} < y_{H_2} < y_{H_3} < x_H$

FIG. 21. THE SCHEMATIC REPRESENTATION OF THE INCREASE IN THE CONCENTRATION OF THE IONS DISSOCIATED BY THE COLLOID (*z*) AND THE DECREASE IN THE CONCENTRATION OF THE FREE IONS OF THE SOLUTION (*y*), IN THE DIRECTION OF THE SURFACE OF THE PARTICLES

Now, it is obvious that

$$y_{H_1} + z_{H_1} > y_{H_2} + z_{H_2} > y_{H_3} + z_{H_3} > x_H;$$

hence the H ion concentration of the suspension or the gel must be higher than that of the filtrate or of the outside solution.

It is also obvious that

$$y_{H_1} < y_{H_2} < y_{H_3} < x_H$$

hence if, by means of increasing pressure, we extract successive portions of the micellar liquid we should find a progressive decrease in the H-ion concentration. The H ions belonging to the micelle are obviously not removable.

As shown in a previous publication (13) in the case of *electropositive colloids*, in which the micellar atmosphere consists of anions, the suspension or the moist gel must react less acid than its extract, but here again the concentration of any ion in the expressed liquid should become smaller at high pressures.

The solution removed from the slightly acidified bentonite gel was found to have a higher pH the higher the pressure, a difference of about 0.2 pH being observed between the solution run off by gravity as compared to the solution obtained in the pressure range between 60 and 70 cm. of mercury. When the bentonite was suspended in a 0.02 *N* NaCl solution instead of water it was found that while the solution which ran off by gravity alone had a concentration of 0.0231 *N*. the latter was reduced to 0.0220 *N*. in the fraction extracted in the pressure range between 60 and 70 cm. of mercury. While this supports the theory it shows that marked differences in concentration are to be expected only at higher pressures. Apparatus permitting an investigation at very much higher pressures are now procured. The results of this work will be reported later.

The author's attention has been called to a recent paper by McCool and Youden (10) which is of the greatest importance to our present subject. They report a considerable decrease in the H- and PO_4-ion concentrations of the solutions removed from peat at high pressures. No definite explanation is offered but they promise to continue the research, which should prove very illuminating.

THE P. D. BETWEEN THE MICELLAR AND THE OUTSIDE SOLUTION

If our postulate that the micellar ion concentration increases rapidly in the direction of the surface of the particles is correct then the potential difference between the gel and the outside solution should show an inverse relationship to the water content of the gel. By the Nernst equation the P.D. is

$$\frac{R\ T}{F}\ ln\ \frac{(y + z)}{x} = \frac{R\ T}{F}\ ln\ \frac{x}{y}$$

The test might meet with difficulties but an attempt to investigate this relationship is now being inaugurated. The plan is to work with the following chain: calomel electrode-saturated KCl—outside equilibrium solution—gel at various water content—saturated KCl—calomel electrode.

DISCUSSION AND SUMMARY

The subject might be briefly recapitulated by the following statements:

The combined or constitutional water represents the internal water of the material in combination with the individual molecules in the form of hydrous oxides of silicon, aluminum, iron, and their combinations.

The molecularly attracted water is represented by the hygroscopic moisture. This water is held by powerful forces which are manifest by the heat of wetting and by a contraction of volume. This water exists in a very thin film which apparently does not exceed 4 or 5 $\mu\mu$ in thickness, which corresponds to the range of molecular attraction. The quantity of water thus adsorbed by the soil is a function of the surface and is independent of the nature and quantity of exchangeable cations. Where a relationship between the heat of wetting on the one side and the composition and exchange capacity on the other side was observed it must be remembered that the particle size and hence the surface varied accordingly (2). The lower heat of wetting reported by Pate (17) for Na-saturated colloids may be ascribed to the slowness with which such materials wet.

Soil colloids imbibe greatly varying quantities of water depending upon:

(a) their composition or more directly upon their exchange capacity;
(b) the nature of the exchangeable cations;
(c) the charge of the particles;
(d) the position of their isoelectric point if amphoteric or
(e) their ultimate pH, i.e., the strength of their acid group;
(f) the concentration of free electrolytes and the valence of the ions, both according to the Donnan equilibrium.

This imbibition is obviously therefore a function of an ionic condition of the colloid, in other words, of a colloidal ionogen. It is an osmotic imbibition brought about by the dissociation of diffusible ions by the colloidal particle. This forms the colloidal micelle consisting of the particle, the ion atmosphere, and the osmotically imbibed water.

The relationship between the applied pressure and the water content indicates that the concentration of the micellar ions increases rapidly in the direction of the surface. This theory leads to the following deductions:

The free electrolyte must be more concentrated in the outside than in the inside or micellar solution. The pH of a negative suspension must be lower than that of its extract.

The pH of a positive suspension must be higher than that of its extract.

The concentration of any ion in the solution removed from a colloid at high pressures must be lower than its concentration in the solution filtered off without pressure.

The potential difference between the gel and its extract must increase as the water content of the gel decreases.

Some of these deductions have been verified.

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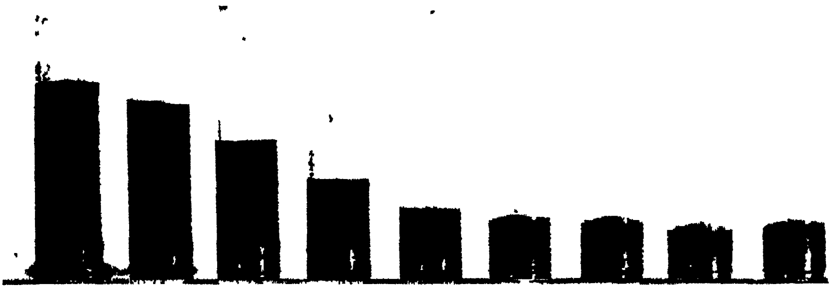
PLATE 1

INFLUENCE OF Na AND Ca UPON THE SWELLING OF THE SHARKEY SOIL COLLOID



Na.	100	75	50	40	30	20	10	5	0
Ca.	0	25	50	60	70	80	90	95	100
cc.	7.20	6 50	5 08	3 20	2.30	2 10	2 00	1.95	1.86

A QUANTITATIVE STUDY OF THE MICROÖRGANIC POPULATION OF A HEMLOCK AND A DECIDUOUS FOREST SOIL

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The research which has been done in the field of soil bacteriology is comparatively recent, falling within the last half century. Among the quantitative investigations are those in which the organisms have been studied in their relation to depth, moisture, hydrogen-ion, concentration and seasonal effects. The attention has mainly been directed toward agricultural soils because of their economic importance; and it is only recently that forest soils have received much notice. It seems logical to the writer, however, that forest soils, of a virgin nature, might contribute more to our knowledge of soil processes, than those under cultivation; for in the case of virgin soils, microbiological processes have, in a sense, reached an equilibrium. This equilibrium is not broken by plowing, changing of crops, addition of fertilizer, or any of the numerous practices of agriculture which tend to upset or disturb the native microörganic reactions; and thus it would seem to furnish more natural conditions than are possible with cultivated soils.

METHODS

An attempt has been made in the present work to examine and compare certain quantitative and general characteristics of the top and subsoils of a hemlock and a deciduous hardwood forest. The "topsoil" includes the three layers generally known as "litter," "fungous," and "true humus" layers; while the "subsoil" is synonymous with the first layer of "mineral soil." Through the courtesy of the New York Botanical Garden[2] samples were collected once a month over a period of 12 months, from January, 1930, to June, 1930, and from October, 1930, to March, 1931, inclusive, omitting the summer months of July, August, and September.

The hemlock and hardwood forests studied are virgin forests on the grounds of the New York Botanical Garden in New York City. They have been uncut and free from fire for over 100 years (47). The hemlock forest where collections were made, consists of approximately pure stands of hemlock; the deciduous hardwood forest is the typical climax forest for this region of North America with beech, sugar maple, white oak, witch hazel, and dogwood as the principal vegetation.

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[1] This work was done in the Department of Botany, Barnard College, Columbia University, under the direction of Dr. Cornelia L. Carey, to whom the writer is indebted for her suggestion of the problem and her interest and guidance throughout the work.

[2] The writer wishes to thank Dr. Marshall A. Howe and Mr. Percy Wilson of the New York Botanical Garden for their cordial coöperation and assistance in the collection of samples.

The soils studied were sent to the U. S. Department of Agriculture for typing. According to this analysis all of the soils are "a very fine sandy loam," "almost a loam," and closely akin to, if not identical with, the Gloucester loam of this vicinity (70). The results of this analysis are given in table 1.

In general, the methods for soil analysis as set forth by Fred and Waksman (81), and by Waksman (78) were followed in this work. Soil was collected from exactly the same locations each month; all collections were made in sterile cans and sterile trowels were used. Soils were sieved through sterile 3-mm. sieves, and sodium albuminate and synthetic acid media (81) were employed.

TABLE 1

Physical texture

TYPES OF SOILS	COARSE SANDS, 2–0.5 MM.	MEDIUM TO FINE SANDS, 0.5–0.10 MM.	VERY FINE SAND, SILT CLAY, < 0.10 MM.	ACIDITY*	ORGANIC MATTER
	per cent	*per cent*	*per cent*	*pH*	*per cent*
Hemlock topsoil	4	17	79	3.80	50
Hemlock subsoil	6	25	69	4.17	7
Deciduous topsoil	6	23	71	4.79	16
Deciduous subsoil	6	21	73	4.50	8

* The pH given in this analysis is somewhat lower than that obtained by the writer, and is no doubt due to the unavoidable time elapsing between the collection of the samples and their analysis, as the writer has found that a storage of only 24 hours of the hemlock topsoil may change the hydrogen-ion concentration from 5.1 to 4.3. This change is due, in all probability, to the activity of the organisms.

The hemlock topsoil and subsoil were plated out a few hours after collection; likewise, 20-gm. samples of all soils were weighed for moisture percentage determinations and the hydrogen-ion concentration of each soil was taken on the day of collection. The plating of the deciduous topsoil and subsoil could not be carried out, for lack of time, until the following morning (18-hour storage) but since this routine was exactly similar from month to month it was considered that conclusions on the relative numbers of organisms would be valid.

Plates were incubated at room temperature in the dark for one week, counts being made on the third, fifth, and seventh days, and the numbers being expressed on the basis of 1 gm. of soil dried to constant weight at 100°C. The hydrogen-ion concentration was taken on a soil extract made by shaking 5 gm. of soil in 50 cc. of distilled water and filtering through two thicknesses of filter paper. Brom cresol green was used as indicator. The moisture holding capacity was found by saturating a given amount of oven-dry soil with a known quantity of distilled water, and determining the amount of water absorbed. The temperature data given in the tables were obtained from the U. S. Department of Agriculture Meteorological Data for New York City (71). Both the mean mean monthly temperature and the average of the mean daily temperature for the five days previous to soil collections were used to determine whether any correlation between the temperature and the total numbers of organisms could be distinguished.

DISCUSSION AND RESULTS

Moisture

As may be seen in tables 2 to 5 the lowest moisture content of the soil corresponds to the lowest count of bacteria, except in the hemlock subsoil where the count remained low even after the moisture had increased. The same is true for the fungi in the two deciduous soils, but is not as evident in the hemlock soils. The actinomycetes show a corresponding drop with lack of moisture in both hemlock soils, but some irregularity in the deciduous soils. Drought, therefore, seems to be a limiting factor. It is of interest in this connection to note that the region of collection (as well as the whole country) suffered many weeks of drought previous to the fall (1930) collections. The fungi seem more resistant to low water content than the bacteria and actinomycetes, for although they show a drop in numbers, it is not such a great one as that exhibited by the bacteria and actinomycetes. In general, the reverse statement is true—that at times when the moisture content is higher, the count of the bacteria and actinomycetes is higher also. Here again the numbers of fungi do not show any marked tendency to follow the moisture curve.

These two statements are in accord with the results of Hiltner and Störmer (36), Kruger and Heinze (41), Engberding (18), Conn (11, 12, 13), Russell and Hutchinson (59), Harder (35), Lochhead (43), Snow (62, 63, 64), and Dixon (16, 17); and although Feher and Sommer (27), and Feher (22, 23, 24, 25) do not draw this conclusion, their data as presented would tend to support the foregoing with respect to forest soils.

Fränkel (30), Remy (55), Fischer (28), Brown and Smith (9), Given and Willis (31), Waksman (75), Brown and Halverson (8), Cutler, Crump, and Sandon (15), Erdmann (19), and Newton (49), on the other hand, find no particular correlation between the moisture percentage and the numbers of organisms.

Freezing

The effects of freezing on the numbers of organisms may be seen from tables 2 to 5. On the whole, there was an increase at the beginning of a frozen period (although this is not apparent in the case of the fungi), and the numbers remained higher than the average throughout a frozen period. In general, then, the numbers at this time were high, but not the highest of the year.

TABLE 2

Hemlock topsoil

DATE (1930–1931)	BACTERIA†	FUNGI†	ACTINOMYCES†	pH‡	WATER	MONTHLY RAIN	TEMPERATURE AVERAGE OF 5 DAYS	MEAN MEAN MONTHLY TEMPERATURE
					per cent	*inches*	*°F.*	*°F.*
*January	2,630,000	6,900,000	400,000	....	50.0	2.58	47.8	33.6
*February	3,437,500	4,500,000	412,500	4.30	60.0	3.40	34.8	37.0
March	2,529,400	4,302,900	105,880	5.10	57.5	2.16	46 0	39.8
April	6,666,600	4,731,100	215,050	4.70	53.5	2.01	41.8	47.6
May	2,164,920	3,092,740	164,940	4.70	51.5	2.89	59.4	62.6
June	4,905,600	3,547,100	188,675	4.85	47.0	2.92	46.8	73.0
October	868,050	3,506,930	8,845	5.15	28.0	1.76	65.2	62.1
November	1,200,850	2,798,850	55,425	5.10	41.0	5.43	54.4	52.1
December	1,237,185	3,092,740	61,855	5.10	51.5	2.65	35.2	41.3
*January	6,185,530	3,814,415	206,180	5.60	51.5	2.43	32.4	40.5
*February	2,250,000	3,825,000	75,000	5 40	60.0	2.44	29.6	42.1
March	2,620,000	3,273,800	28,450	5.30	58.0	4.74	36 4	40.5

*Soil frozen.

† Counts of organisms represent numbers per gram dry soil.

‡ pH taken after a 6-hour storage except in March, 1930, where a 24-hour storage was unavoidable.

TABLE 3

Hemlock subsoil

DATE (1930–1931)	BACTERIA†	FUNGI†	ACTINOMYCES†	pH‡	WATER	MONTHLY RAIN	TEMPERATURE AVERAGE OF 5 DAYS	MEAN MEAN MONTHLY TEMPERATURE
					per cent	*inches*	*°F.*	*°F.*
*January	2,483,600	686,300	196,000	....	23.5	2.58	47.8	33.6
*February	2,030,000	530,825	150,370	4.55	33.5	3.40	34.8	37.0
March	1,830,100	577,460	140,800	5.05	29.0	2.16	46.0	39.8
April	2,922,450	706,120	122,440	4.70	26.5	2.01	41.8	47.6
May	191,250	81,960	0	4.90	26.8	2.89	59.4	62.6
June	606,450	429,675	90,300	4.90	22.5	2.92	46.8	73.0
October	349,715	323,430	14,860	5.20	12.5	1.76	65.2	62.1
November	252,940	235,295	14,705	5.10	15.0	5.43	54.4	52.1
December	278,430	343,790	39,215	5.10	23.5	2.65	35.2	41.3
*January	2,202,610	143,790	91,500	5.50	23.5	2.43	32.4	40.5
*February	623,265	373,960	42,380	5.40	27.8	2.44	29.6	42.1
March	994,595	236,480	148,645	4.90	26.0	4.74	36.4	40.5

* Soil frozen.

† Counts of organisms represent numbers per gram dry soil.

‡ The pH is taken after a 6-hour storage in every case except for March, 1930, where a 24-hour storage was unavoidable.

TABLE 4

Deciduous topsoil

DATE (1930–1931)	BACTERIA†	FUNGI†	ACTINOMYCES†	pH‡	WATER	MONTHLY RAIN	TEMPERATURE AVERAGE OF 5 DAYS	MEAN MEAN MONTHLY TEMPERATURE
					per cent	*inches*	*°F.*	*°F.*
*January	8,360,900	1,278,100	188,000	....	33.5	2.58	47.8	33.6
*February	20,543,200	3,260,900	761,000	4.80	54.0	3.40	34.8	37.0
March	7,086,600	2,047,200	472,400	5.05	36.5	2.16	46.0	39.8
April	9,000,000	1,444,430	555,550	4.80	32.5	2.01	41.8	47.6
May	2,991,100	590,840	295,420	4.80	32.3	2.89	59.4	62.6
June	5,518,200	1,138,660	233,575	5.05	31.5	2.92	46.8	73.0
October	858,800	900,000	76,470	5.20	15.0	1.76	65.2	62.1
November	2,200,000	3,040,000	33,330	5.15	25.0	5.43	54.4	52.1
December	1,566,150	1,786,740	73,530	5.20	32.0	2.65	35.2	41.3
*January	4,223,850	1,044,750	49,250	5.70	33.0	2.43	32.4	40.5
*February	5,811,940	2,478,600	213,675	5.50	41.5	2.44	29.6	42.1
March	7,445,250	1,587,600	620,435	5.30	31.5	4.74	36.4	40.5

* Soil frozen.

† Counts of organisms represent numbers per gram dry soil.

‡ The pH is taken after a 6-hour storage in every case except for March, 1930, where a 24-hour storage was unavoidable.

TABLE 5

Deciduous subsoil

DATE (1930–1931)	BACTERIA†	FUNGI†	ACTINOMYCES†	pH‡	WATER	MONTHLY RAIN	TEMPERATURE AVERAGE OF 5 DAYS	MEAN MEAN MONTHLY TEMPERATURE
					per cent	*inches*	*°F.*	*°F.*
*January	5,986,000	650,350	251,745	....	28.5	2.58	47.8	33.6
*February	5,864,600	548,900	248,120	4.60	33.5	3.40	34.8	37.0
March	5,310,000	655,170	172,400	5.00	27.5	2.16	46.0	39.8
April	2,000,000	866,650	166,665	4.70	25.0	2.01	41.8	47.6
May	3,809,500	721,090	292,515	4.90	26.5	2.89	59.4	62.6
June	6,352,930	1,124,160	209,150	5.05	23.5	2.92	46.8	73.0
October	647,725	250,000	51,140	5.25	12.0	1.76	65.2	62.1
November	807,020	301,755	70,175	5.10	14.5	5.43	54.4	52.1
December	2,373,325	620,000	153,330	5.00	25.0	2.65	35.2	41.3
*January	1,792,245	553,545	116,125	5.50	22.5	2.43	32.4	40.5
*February	1,956,330	253,520	84,510	5.50	29.0	2.44	29.6	42.1
March	913,905	105,960	13,245	5.30	24.5	4.74	36.4	40.5

* Soil frozen.

† Counts of organisms represent numbers per gram dry soil.

‡ The pH is taken after a 6-hour storage in every case except for March, 1930, where a 24-hour storage was unavoidable.

The increase at a time of freezing would tend to confirm the findings of Conn (11, 12, 13), Weber (82), Brown and Smith (9), Given and Willis (31), Harder (35), Waksman (75), Noyes (50), Vass (74), and Brown and Halverson (8), although these investigators differ widely in their theories to account for this increase. Lochhead (43) found only a slight increase in Canadian soil which was frozen consecutively for four or five months, while Vanderleck (72), who also studied Canadian soil under the same conditions, failed to find any noticeable increase in bacterial numbers.

It would seem, then, from the experimental data, that soils which are continuously frozen for months at a time, do not show a marked increase in numbers, or if they show it at the beginning of such a frozen period, the numbers eventually drop to a normal level and remain there. On the other hand, soils which are subject only to north temperate climatic conditions, such as those studied here, and which undergo thawing and freezing rather frequently during the winter months, may exhibit increases in numbers during their frozen periods.

Hydrogen-ion concentration

From data in tables 2 to 5 it will be seen that the small changes in the hydrogen-ion concentration have no apparent relationship to the numbers of organisms present in the soils. Feher (22, 23, 24, 25) and Jensen (38), both working with forest soils of a virgin nature, have found a similar lack of correlation, although Jensen finds a strong positive correlation between the numbers of fungi and of bacteria plus actinomycetes when the hydrogen-ion concentration is 5.5 or below. Bokor (3), working on German forest soils, reports that the total number of bacteria depends chiefly on the hydrogen-ion concentration of the soil, and if this remains constant, the numbers increase in proportion to the organic matter present.

Seasonal effects

The maxima for all three types of organisms seem to come at the same time of year. If the high numbers found at the frozen periods are not considered as true maxima, but as due to some physical cause (such as the breaking up of the clumps, or the rise of organisms with that of the capillary water on freezing), then there is only one maximum. This comes in April at the beginning of the growing season (tables 2 to 5). Two exceptions are to be noted: (*a*) In the deciduous subsoil the rise in bacterial numbers comes later; and (*b*) in the deciduous topsoil the fungi show no apparent April peak. If, as others think, the increase at frozen periods is due to an actual reproductive increase in the numbers of organisms, then two maxima may be seen; that during the frozen period, and the one in April. Interpretation of the increase on a purely mechanical basis seems more logical to the writer, but no definite evidence for either view is available from the present data. It is interesting to note that the June count, in every case except two, leaves the numbers increasing (tables 2 to 5). It is most unfortunate that the July, August, and September counts could not be made, as investigators who have worked during the summer months on forest soils, Rivas (56), in this country, and Feher (22, 23, 24, 25), in Germany, have found the greatest number of organisms in July or August, and the rise to this peak has begun, in nearly every case, in the month of June. In a number of cases both workers found small peaks in April but these were insignificant in comparison to the July and August maxima.

Temperature

The air temperature has been considered in tables 2 to 5 from two standpoints. In one case, an average of the mean daily temperature for five days previous to collection was taken, in an effort to see whether this might have any bearing on the numbers of organisms at that particular time. In the other case, the mean mean of the monthly temperature was used to determine whether any general effect of the temperature was noticeable. To compare with this, no soil temperatures could be obtained, but in the work of Feher (22, 23, 24, 25) and of Feher and Bokor (26) on forest soils, the soil temperatures and air temperatures are seen to correlate closely, their curves running parallel with only a few degrees difference, so that this would probably be true of any forest soil.

Fabricius and von Felitzen (20), Given and Willis (31), and Brown and Halverson (8) found a definite correlation between the numbers of bacteria and the temperature, in non-forest soils. This was confirmed for forest soils by Feher (22, 23, 24, 25) and Rivas (56).

No such correlation between numbers of organisms and temperature was found in the present work, as may be seen in tables 2 to 5. Other investigators whose findings agree with those given in the tables above are: Fränkel (30), Hiltner and Störmer (36), Engberding (18), and Cutler, Crump, and Sandon (15). Dixon (16, 17) has found (in Australian bush soils) that moisture is more of a controlling factor than temperature as far as the numbers of organisms are concerned, but that a low temperature decreases the numbers.

Depth

Because the topsoil (consisting of the litter, fungus, and true humus layers) and subsoil (or first mineral layer) could be so easily distinguished, the samples were taken according to layers rather than to any definite depth, but the total depth at no time exceeded 35 cm. Tables 2 to 5 reveal that with but few exceptions the numbers of organisms in the two subsoils are less than those in their corresponding topsoils. From this, then, we might conclude that there is a decrease in the numbers of bacteria, fungi, and actinomycetes with an increase in the depth. This is a well-established fact, but the decrease in numbers in the soils here studied may also be due, in part, to the great difference in the chemical nature of the topsoil and its corresponding subsoil. It would seem that since the topsoil has so much more organic matter, and hence more food and energy sources for the organisms, than its corresponding subsoil, this, rather than a few centimeters difference in depth would cause the drop in the numbers of the different types of microörganisms.

In tables 2 to 5 the moisture content and hydrogen-ion concentration of the various soils are given. The hemlock topsoil, which is the richest in organic matter, has also the highest percentage of moisture in every case; the deciduous topsoil, the next richest in organic matter, has the next highest moisture percentage; and the deciduous subsoil and Hemlock subsoil, having about the same amount of organic matter—much less than the two topsoils—, also have about the same percentage of moisture. (For data on organic matter see table 1.) Thus, the amount of organic matter present seems to have considerable to do with the water holding capacity of the soil.

There is no great difference in hydrogen-ion concentration between any of the four soils, all of them, as soils go, being quite acid, having a range of 4.55–5.7. This acidity is true, in general, for forest soils, and may be partly due to the fungous flora which is abundant in such soils (though, conversely, the fungi flourish because they can stand acidity), and partly to the character of the organic residues in the topsoils. Agricultural soils, for example, have a hydrogen-ion concentration around 7.0, and are richer in bacteria and poorer in fungi than forest soils.

As for the depths of the hemlock and deciduous topsoils, the transition between the two layers (top and subsoil) is much more abrupt in the hemlock soil. Here the topsoil is definitely of a peaty nature, only a few centimeters thick. Of the three layers composing it, the true humus layer is very small—a fraction of a centimeter in most cases—, and the fungous layer predominates. The deciduous topsoil merges with the subsoil more gradually, is deeper, on the whole, than the hemlock topsoil, and is of a "moldy" (leaf mold) rather than a peaty nature. The humus layer is several millimeters thicker than that of the hemlock topsoil, but the fungous layer is not as deep. The layer of litter, though deeper, is less compact than the hemlock litter, so probably does not represent much more material, except after the autumn leaf fall. The two topsoils correspond to the "mull" (mold) and "torf" (peat) soils described for Danish forest soils by Muller (57, p. 70).

Bacteria

Table 6 shows a decided parallelism between the numbers of the bacteria in the hemlock topsoil and hemlock subsoil; and some similarity in the numbers in the deciduous topsoil. The deciduous subsoil, however, fails to show any particular correlation with the other three.

Certain climatic factors, such as air temperature and wind velocity, would have affected these soils similarly. The moisture holding capacity of the soils, however, is not the same. Upon calculation (pp. 326–327) the total moisture holding capacity was found to be as follows: hemlock topsoil 105 per cent, hemlock subsoil 73 per cent, deciduous topsoil 85 per cent, deciduous subsoil 73 per cent. Considering the optimum moisture conditions to be 70 per cent of the maximum, as is generally accepted, we have the optimum as: hemlock topsoil 73.3 per cent, hemlock subsoil 51.1 per cent, deciduous topsoil 59.5 per cent, and deciduous subsoil 51.1 per cent. A comparison of these figures with those for percentage of organic matter (table 1) shows that the soils with the most organic matter hold the most moisture. It is evident that the hemlock topsoil with its average moisture for the year of 50.8 per cent comes nearest to filling optimum moisture conditions as far as the organisms are concerned; deciduous topsoil with a year's average of 33.19 per cent comes next; and the two subsoils (deciduous subsoil with an average of 29.2 per cent, and hemlock subsoil with 24.17 per cent) are about equal in this respect.

TABLE 6

Bacteria†

DATE (1930–1931)	HEMLOCK TOPSOIL	HEMLOCK SUBSOIL	DECIDUOUS TOPSOIL	DECIDUOUS SUBSOIL
*January	2,630,000	2,483,600	8,360,900	5,986,000
*February	3,437,500	2,030,000	20,543,200	5,864,600
March	2,529,400	1,830,100	7,086,600	5,310,000
April	6,666,600	2,922,450	9,000,000	2,000,000
May	2,164,920	191,250	2,991,100	3,809,500
June	4,905,600	606,450	5,518,200	6,352,930
October	868,050	349,715	858,800	647,725
November	1,200,850	252,940	2,200,000	807,020
December	1,237,185	278,430	1,566,150	2,373,325
*January	6,185,530	2,202,610	4,223,850	1,792,245
*February	2,250,000	623,265	5,811,940	1,956,330
March	2,620,000	994,595	7,445,250	913,905

* Soil frozen.

† Counts of organisms represent numbers per gram dry soil.

Now since it is the deciduous subsoil (table 6) which fails to show any parallelism to the other three, and since both the deciduous subsoil and the hemlock subsoil have about the same amount of moisture available to the organisms, we may conclude that moisture, at least, is not responsible for the variations of the deciduous subsoil. Neither is the hydrogen-ion concentration different enough in this soil to be looked upon as a cause.

We must look, then, to some other factor to account for its behavior. This might be: (*a*) a difference in the available food supply for the bacteria in the deciduous subsoil, or (*b*) a difference in the influence of higher plants on the growth of the bacteria in the deciduous subsoil as compared with that in the other soils.

There are in this paper, however, no experimental data to confirm either assumption. They are simply submitted as possible suggestions to account for the dissimilarity of this soil when compared with the others.

The ability of microörganisms to carry on their vital activities depends, as is well known, upon a number of factors, some of which have already been mentioned. There are probably many others of importance about which we have, as yet, little knowledge. Our information is not complete as to the influence of higher plants upon the microörganisms of the soil. The dying of the cells of higher plants affects, of course, the available food supply of the microörganisms by the release of dead organic and inorganic material. Besides this, the plant roots may secrete substances which may hinder or help the organisms. Hansteen-Cranner (34) has shown that plants under ordinary conditions give off from their root cells soluble and insoluble lipoid substances termed "phosphatides." These differ with the plant species and may inhibit or stimulate the microörganisms in that vicinity. Melin (46) has reported a substance which is probably identical to the "phosphatides" of Hansteen-Cranner, from the roots of pine and other coniferous trees. This material has a stimulating action on the growth of certain mycorrhizal fungi. Other investigators have reported on the stimulation of bacterial activity by plant roots; and the excretion of certain salts, organic and inorganic acids, and a number of different sugars has likewise been demonstrated (44, 45, 79). Starkey (65) has made the interesting suggestion that the growth of the higher plants often causes an unequal distribution of the numbers of microörganisms, and that it "may be a major factor in the seasonal fluctuation of the microorganisms in the soil where temperature and moisture do not seem to be related."

As far as the bacteria are concerned, the numbers seem to run parallel to each other in all except the deciduous subsoil. This lack of parallelism cannot be accounted for by any of the factors examined in the present work, but it is suggested that it might be due to a difference in the available food supply of the organisms, or, perhaps, to the influence of the higher plant roots growing at the locations of the soil collections.

It is evident from table 6 that there are, on the average, a greater number of bacteria in the deciduous topsoil than in any other soil. An average for the 12 months of all the bacterial numbers (table 9) shows that the deciduous topsoil has about twice as many bacteria as the deciduous subsoil or the hemlock topsoil (which two soils have about equal numbers) and almost six times as many as the hemlock subsoil. This would suggest that the bacteria play a more active rôle in digestion in the deciduous topsoil than in any of the other soils studied.

Actinomycetes

From table 7, which shows the numbers of actinomycetes, it is evident that the numbers in the two topsoils run somewhat parallel to each other from January to June, 1930, and exactly counter to each other (i.e. if the numbers increase from one month to the next in one soil, they decrease in the other, and vice versa) from October, 1930, to March, 1931. The numbers in the subsoils, however, which run counter to each other from January to June, 1930, run nearly parallel from October, 1930, to February, 1931, except that the greatest number in the deciduous subsoil comes one month before that in the hemlock subsoil in the early winter. Whether this is merely accidental, or whether there are complicated factors at work here, is a problem for further study.

There is, however, an interesting point of comparison between the data for the actinomycetes and that presented for the bacteria. An average of the year's counts (table 9) reveals that, almost exactly as in the case of the bacteria, the deciduous topsoil has approximately twice as many actinomycetes as the deciduous subsoil or hemlock topsoil and more than three times as many as the hemlock subsoil, thus giving almost the same comparative relationship as before. It would seem, then, that as in the case of the bacteria, the actinomycetes were more actively concerned with digestion processes in the deciduous topsoil than in the other soils.

TABLE 7

Actinomyces†

DATE (1930–1931)	HEMLOCK TOPSOIL	HEMLOCK SUBSOIL	DECIDUOUS TOPSOIL	DECIDUOUS SUBSOIL
*January	400,000	196,000	188,000	251,745
*February	412,500	150,370	761,000	248,120
March	105,880	140,800	472,400	172,400
April	215,050	122,440	555,550	166,665
May	164,940	0	295,420	292,515
June	188,675	90,300	233,575	209,150
October	8,845	14,860	76,470	51,140
November	55,425	14,705	33,330	70,175
December	61,855	39,215	73,530	153,330
*January	206,180	91,500	49,250	116,125
*February	75,000	42,380	213,675	84,510
March	28,450	148,645	620,435	13,245

* Soil frozen.

† Counts of organisms represent numbers per gram dry soil.

Fungi

From table 8, which shows the numbers of fungi in the four soils, it may be seen that there is some parallelism between the numbers for the hemlock subsoil and those for the deciduous subsoil, except in two points, November, 1930, and February, 1931. It is also readily seen that the numbers of fungi in these two subsoils are approximately equal.

On the other hand, it is evident that the numbers in the hemlock topsoil far exceed those of any of the other four soils, whereas those of the deciduous topsoil lie in an intermediate position. The numbers in the two topsoils show very little similarity to each other, or to those of the two subsoils. Considering the entirely different chemical nature of the topsoils, due to the different types of vegetation they support, and hence the difference in their organic residues, this is not surprising. The two subsoils, on the other hand, contain about equal amounts of organic matter, and it is possible that the close agreement of the numbers of fungi in the subsoils might be due to this fact, although further work would have to be done to determine this.

The fungous activity is, then, far greater in the hemlock topsoil than in any of the other soils studied; it is next greatest in the deciduous topsoil, and least in the two subsoils. The numerical relationship in the case of the fungi, is not comparable to that of the actinomycetes and bacteria. The hemlock topsoil has about twice as many fungi, on the average, as the deciduous topsoil, seven times as many as the deciduous subsoil, and ten times as many as the hemlock subsoil (table 9).

TABLE 8

Fungi†

DATE (1930–1931)	HEMLOCK TOPSOIL	HEMLOCK SUBSOIL	DECIDUOUS TOPSOIL	DECIDUOUS SUBSOIL
*January	6,900,000	686,300	1,278,100	650,350
*February	4,500,000	530,825	3,260,900	548,900
March	4,302,900	577,460	2,047,200	655,170
April	4,731,100	706,120	1,444,430	866,650
May	3,092,740	81,960	590,840	721,090
June	3,547,100	429,675	1,138,660	1,124,160
October	3,506,930	323,430	900,000	250,000
November	2,798,850	235,295	3,040,000	301,755
December	3,092,740	343,790	1,786,740	620,000
*January	3,814,415	143,790	1,044,750	553,545
*February	3,825,000	373,960	2,478,600	253,520
March	3,273,800	236,480	1,587,600	105,960

* Soil frozen.

† Counts of organisms represent numbers per gram dry soil.

From data in tables 6, 7, and 8 it is apparent that the fungi far outnumber the bacteria and actinomycetes in the hemlock topsoil. The decomposition of organic matter (hemlock needles, twigs etc.) by fungi, is evidently predominant in the hemlock topsoil. This decomposition is thorough and quite rapid, for, as we have said, the topsoil is only a few centimeters deep. The fungi are apparently more successful in the digestion of the particular organic residues found in the hemlock litter than the bacteria are, and hence are more abundant in this soil than the latter. The bacterial numbers on the other hand, predominate in the deciduous topsoil, and probably are responsible for most of the decomposition there. Digestion, no doubt is aided by the fungi, but is largely accomplished by the bacteria with the help of the actinomycetes. The actinomycetes are not numerous in any of the soils, undoubtedly because of their acid character; neither, if we may judge by the numbers, do these organisms accomplish as much work in the two topsoils as do the fungi and bacteria. Most of the actual decomposition takes place in the "rich" topsoils, but as is shown in tables 6, 7, and 8, the bacteria are the most important agents in the digestion of the material left in the subsoils. They are assisted in this by both the fungi and the actinomycetes.

In order to determine whether there was any constancy or predominance of the types of fungi occurring in the soils from month to month, the synthetic acid medium plates were examined at the end of each count and the types of organisms which predominated were noted.

The species of Penicillium whose conidial stages were different shades of green (macroscopically) could be easily distinguished from species of Trichoderma and Mucor. The typical gray-green colonies of Citromyces were counted from month to month, as well as those of the rest of the Penicillium group mentioned. Since these counts in every case were based purely on macroscopic observations, they cannot be considered more than very roughly accurate. Colonies of each type of fungous were subcultured and examined microscopically from time to time to determine whether macroscopic observations had been valid; and they were found to be so in nearly every case.

TABLE 9

Average of numbers for the 12 months

Numbers given per gram dry soil

	HEMLOCK TOPSOIL	HEMLOCK SUBSOIL	DECIDUOUS TOPSOIL	DECIDUOUS SUBSOIL
Bacteria	3,057,960	1,230,450	6,300,500	3,151,130
Fungi	3,948,800	389,100	1,716,490	554,260
Actinomyces	160,190	87,600	297,690	152,430
Citromyces	417,460	25,330	12,090	4,945
Penicillium group excluding Citromyces	553,730	23,400	79,310	35,900

Hemlock topsoil

Average of Penicillium group excluding Citromyces for March, 1930, January, February, March, 1931	631,750
Average of Penicillium group excluding Citromyces for other eight months	376,285
Average of Citromyces group alone for March, 1930, January, February, March, 1931	576,620
Average of Citromyces group alone for other eight months	233,515

Tables 10 and 11 show the comparative numbers for Citromyces and Penicillium in the four soils. These figures run parallel to those for the total numbers of fungi; namely, the hemlock topsoil has the greatest number, the deciduous topsoil the next greatest, and the two subsoils the least, although about equal numbers.

By taking the averages for the 12 counts and comparing them with the averages of the total numbers of fungi (table 9) for the particular soils in question, it is seen that the Penicillium group, including Citromyces, forms a much higher percentage of the total numbers of organisms in the hemlock topsoil than in any of the other three soils. Possibly this group has some special rôle in the digestive processes in the Hemlock topsoil, although there is in the present data no proof of this.

TABLE 10

Total numbers of Citromyces per gram dry soil

DATE (1930–1931)	HEMLOCK TOPSOIL	HEMLOCK SUBSOIL	DECIDUOUS TOPSOIL	DECIDUOUS SUBSOIL
*January	....	....	......	......
*February	....	....	......	......
March	1,294,100	42,260	......	......
April	307,530	156,460	26,670	20,000
May	212,370	4,100	22,160	8,850
June	558,490	2,970	8,760	1,700
October	388,890	11,430	0	0
November	277,120	5,880	......	6,610
December	123,710	785	0	0
*January	309,280	395	4,480	1,940
*February	465,000	2,080	27,350	2,110
March	238.090	27.025	7.300	3.310

* Soil frozen.

TABLE 11

Total numbers of Penicillium group per gram dry soil

DATE (1930–1931)	HEMLOCK TOPSOIL	HEMLOCK SUBSOIL	DECIDUOUS TOPSOIL	DECIDUOUS SUBSOIL
*January	....	....	....	....
*February	....	....	....	....
March	541,200	18,310	....	....
April	178,500	6,800	71,110	20,000
May	556,700	8,200	73,860	34,020
June	213,200	5,550	48,180	8,630
October	694,400	5,720	35,300	11,370
November	831,400	13,030	....	175,440
December	536,100	104,580	101,470	13,330
*January	886,600	6,540	128,360	16,780
*February	432,500	18,010	147,010	30,280
March	666.700	47.300	29.200	13.240

* Soil frozen.

From tables 10 and 11 it is apparent that the Penicillium group, including Citromyces, in the hemlock topsoil, form approximately one-fourth of its total fungous population. (See also table 9). In both subsoils their average is roughly one-eighth, whereas in the deciduous topsoil this group forms only one-nineteenth of the total number. Thus it may be seen that in all, except the hemlock topsoil, the Penicillium group with Citromyces forms a rather insignificant portion of the fungous population.

Actual macroscopic counts of the organisms were not begun until March, 1930, but if the average of the numbers of Citromyces for March, 1930, plus January, February, and March, 1931, be compared with those of the rest of the Penicillium group for the same months, it will be seen that in the hemlock topsoil the Citromyces group is to the Penicillium as 576,600 is to 632,000; or roughly, as 1:1. (table 9). Making the same comparison for the other eight months the relationship is: Citromyces to Penicillium group as 233,500 to 376,300; or roughly, 1:1½ (table 9). Thus not only do the actual numbers of these two groups increase during January, February, and March, but the Citromyces group increases proportionally more than the Penicillium group in the hemlock topsoil. This is interesting and may point to some special function of Citromyces at this time of the year. That this increase in numbers is the result of freezing does not seem the explanation, since the numbers of Trichoderma do not increase during this period, but become less.

Not only do the numbers of Trichoderma become less when the Penicillium group increases, but when the Penicillium group has lost its predominance, in the fall months, Trichoderma again increases greatly, and covers, on the average, 7 of the 9 synthetic acid medium plates poured for any one soil, so that at the end of five days they cannot be counted. This would suggest some mutual seasonal relationship between these two groups of fungi (Penicillium and Trichoderma).

Representatives of the Mucorales were very scarce, and never more than three or four colonies appeared on all the plates from any one soil. They were found, sparsely, in every soil, no soil having particularly more than another. To determine whether the synthetic acid medium was unfavorable to their development, plates were poured (in January, 1931) of Cook's agar (8) as well as the synthetic acid medium plates, since Cook's agar is supposed to favor the growth of Mucorales. More specimens of Mucor came up on the synthetic acid plates than on those of Cook's agar, so it would appear that the medium was not the cause of the lack of Mucors. In defense of this point, Waksman (76) has found that forest and uncultivated soils are poor in Mucorales but rich in specimens of Penicillium and Trichoderma. Jensen (38) in examining 18 forest soils in Denmark reports similar findings.

Besides a scarcity of Mucors, there was but one Aspergillus isolated from the plates during the entire study. Since the conidial stage of the species isolated was not green, it was considered valid to eliminate the species of Aspergillus from the counts of the Penicillium group mentioned in the foregoing. A paucity of Aspergillus in soils from this latitude has been found by other investigators. Waksman (77) and Werkenthin (83) both found that soils in the southern part of the United States were abounding in species of Aspergillus, whereas those of the northern part of the country had predominantly species of Penicillium, Mucor, Trichoderma, and Fusarium, but were poor in Aspergillus.

Waksman (77), Takahashi (67), Fasevoli (21), Paine (51), Dixon (16), Swift (66), and Jensen (38) report that soil fungi common to all parts of the world are species of Penicillium (including Citromyces), Trichoderma, Mucor, and Aspergillus. Some of these writers include species of Fusarium, as well.

Citromyces is typical of the hemlock topsoil. The deciduous top and subsoils seemed to have no "typical fungi" (that is, fungi which occurred in abundance on the plates month after month, but which were not found on plates of the other soils). The hemlock subsoil, however, had two forms which were present in abundance at every collection. The first of these was a gray-green Penicillium which was a bright rose color on the under side (species undetermined). There were always numerous colonies of it on the hemlock subsoil plates, but seldom more than one, if any, on the plates of the other three soils. Its significance, if there is any, offers a problem like that of the Citromyces in the hemlock topsoil, and as suggested for Citromyces, may be concerned with soil digestion processes. The other fungus found so persistently on the hemlock subsoil plates was a sterile white mycelium. Such sterile hyphae have been reported by other workers for forest soils, notably by Traaen (69) for Norwegian soils, by Waksman (76) for American soils, and by Jensen (38) for Danish soils. Waksman thinks that these mycelia are probably the hyphae of mycorrhizal fungi. This might very well be the case here, as the sucking roots of the coniferous trees are known to have an abundant mycorrhizal fungous flora. In any event, their presence in the hemlock subsoil is interesting.

The work of identification of many of these fungous forms is, in itself, a considerable undertaking, as a large proportion of the soil fungi belong to the group "Hyphomycetes," and the numerous sterile hyphae present a baffling problem in that they do not produce conidial stages in the laboratory under ordinary conditions. If the latter are mycorrhiza, as suggested, it is possible that, given other conditions, they might be induced to fruit.

SUMMARY

In general, the greatest numbers of bacteria, fungi, and actinomycetes corresponds to the time of greatest soil moisture. The converse is also true.

Freezing of the soil causes a marked increase in the numbers of organisms, but this increase is not so apparent in the case of the fungi. The frozen counts are high, but not the highest of the year.

The slight variations in the hydrogen-ion concentration have no apparent effect on the total numbers of bacteria, fungi, and actinomycetes.

In general, there is some parallelism between the numbers of bacteria and actinomycetes in each soil. It is not so marked in the case of the fungi.

The seasonal maxima for the three types of organisms come in all soils in April, the beginning of the growing season.

The air temperature seems to have no definite relation to the total numbers of bacteria, fungi, or actinomycetes.

There are, in every case, fewer organisms in the samples of subsoils than in their corresponding topsoils. This may be due to the sandy nature of the subsoils, and to their lack of organic matter, or it may be due purely to the greater depth of these collections.

The deciduous topsoil has twice as many bacteria as the deciduous subsoil or hemlock topsoil (which two soils have about equal numbers) and almost six times as many as the hemlock subsoil.

The deciduous topsoil has twice as many actinomycetes as the deciduous subsoil or hemlock topsoil and nearly three times as many as the hemlock subsoil.

The hemlock topsoil has twice as many fungi as the deciduous topsoil; seven times as many as the deciduous subsoil; and ten times as many as the hemlock subsoil.

These figures show that in the peaty hemlock topsoil the molds are responsible for most of the decomposition; whereas in the deciduous topsoil the bacteria are the main agents in the soil digestion processes, and the fungi are in second place according to numbers. These two topsoils contain, of course, the greatest amounts of undecomposed material.

Most of the work of decomposition is completed in the two topsoils, but in the subsoils that which is left to be done is carried on mainly by the bacteria. The actinomycetes and fungi aid in the process but are not the main agents.

The species of Penicillium including Citromyces show the same numerical relationship to each other in the four soils as do the total numbers of fungi; namely, most numerous in the hemlock topsoil, next most numerous in the deciduous topsoil, and least, but about equally numerous, in the two subsoils. These figures are based purely on macroscopic counts of species of Penicillium or Citromyces whose conidial stages appeared green.

The Penicillium group, including Citromyces, are actually and proportionally more numerous in the hemlock topsoil than in the others. This would suggest that they might play some special rôle in the soil processes of the hemlock topsoil.

The ratio of Citromyces to the Penicillium group (not including Citromyces) in the hemlock topsoil is greater for the four months of March 1930, January, February, and March, 1931, than is the ratio of these organisms during the other eight months that the soils were examined. These averages of numbers check with general notes taken on the plates from month to month, and suggest that Citromyces might have some special function in the hemlock topsoil at this season of the year.

At the time of year when the Citromyces-Penicillium are predominant in the hemlock topsoil, species of Trichoderma fade into the background. The reverse is also true. This would suggest some mutual relationship between Trichoderma and the Penicillium group (including Citromyces).

Species of Penicillium (including Citromyces) and Trichoderma seem to form by far the largest part of the fungous population of the four soils studied. This confirms the work of other forest soil investigators.

Members of the order Mucorales and the genus Aspergillus were found to be very scarce in the soils studied. This also confirms the results of other workers on forest soils.

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THE DIURNAL AND SEASONAL CHANGES IN THE SUGAR CONTENT OF THE SAP AND TISSUE OF POTATO PLANTS AS AFFECTED BY SOIL FERTILIZATION[1]

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One of the most interesting phases of the many chemical studies made of plant tissue is the determination of carbohydrates in the various parts of plants. Most of this work has been directed toward determining how carbon assimilation takes place and how its products are transported from the leaves to other parts of the plant, either for immediate or future use.

The object of this experiment was to study the diurnal and seasonal variations in the sugar content of both leaves and stalks of potato plants for the purpose of determining any differences that might occur as a result of the application of commercial fertilizers to soils growing these potato plants under field conditions, and to determine whether any differences could be correlated with the crop yields. The potato was selected because this plant, through its habits of storing carbohydrates, lends itself very well to this type of investigation.

HISTORICAL

Although Liebig (18) in 1832 advanced the first theory of carbon assimilation in plants, and suggested that organic acids were perhaps the intermediate products, the work of Sachs (28) in 1862, which showed that starch is actually produced within the chlorophyll granule, was really the starting point of investigations concerning carbon assimilation in plants. In 1870, von Baeyer (2) advanced his famous formaldehyde theory, but later Brown and Morris (5), Parkin (24) and Davis, Daish, and Sawyer (8), brought forth evidence to prove that sucrose and not glucose is the first sugar of photosynthesis. However, the work of Dixon and Mason (10), Priestly (25), and more recently that of Clements (6, 7), Baly and Davis (3), and Barton-Wright and Pratt (4), may be interpreted as favoring the von Baeyer theory.

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A more specialized field of investigation, that of the carbohydrate-nitrogen ratio in plants, has been investigated primarily by horticulturists. Some of the most noteworthy work is that of Kraus and Kraybill (17), Reid (27), Harvey and Murneek (13), Hooker (14), and Murneek (23), all of whom have found that variations in the carbohydrate-nitrogen ratio are accompanied by variations in the physiological functions of the plant.

Mason and Maskell (20) studied the translocation of carbohydrates in cotton plants and found a lag in the cycle of the maximum carbohydrate movement from leaves to bark.

Janssen and Bartholomew (15, 16) found that the maximum carbohydrate production in plants growing in sand and water cultures occurred with a concentration of 3 p.p.m. of potassium in the solution, but could not obtain consistent correlations in carbohydrate production by the same kind of plants grown on field soils fertilized with different amounts of potassium fertilizer.

Clements (6) found reciprocal relations for nitrogen and carbohydrates in field peas grown in water cultures, and Woo (32) obtained similar results with certain plants grown in the field. Hartwell (12) found that a number of factors associated with retarded growth caused an increase in the starch content of potato vines.

EXPERIMENTAL OUTLINE

Two tiers of fertilized plots were used, each plot being 2 rods by 4 rods, with a check strip 1 rod wide between the two tiers of plots. The following fertilizers were used: 4–16–0, 0–16–8, 4–16–8, and 4–16–12. Each treatment was replicated four times. Samples for analysis were taken from one set of the replications and the remaining three sets were used for obtaining the crop yields. No samples were taken from the 4–16–12 plot, because in some previous work it was found that the yields were almost the same on the plots treated with the 4–16–8 and 4–16–12 fertilizer. The latter treatment was included in the experiment in order further to compare it with the 4–16–8 treatment from the standpoint of its effects on the crop yields.

The experiment field was located about 1½ miles from the laboratory, where the analyses were made. The soil of the experiment field was Hillsdale sandy loam. This soil type is one of the principle moranic soils of south central Michigan, and is well adapted to the growing of potatoes. The fertilizers applied were made from ammonium sulfate, 40 per cent superphosphate, and muriate of potash. They were broadcast by hand at the rate of 750 pounds an acre and worked into the soil with a spring-tooth drag shortly before the potatoes were planted.

WEATHER CONDITIONS

As the season of 1930 was extremely dry, the yield of the potatoes ranged only from 90 to 100 bushels an acre, and the plots showed no differences in yields attributable to the fertilizer treatments. In the years of normal rainfall, however, yields of from 200 to 300 bushels an acre were obtained.

The plots were sampled on July 23, August 12, and September 4. During this period only 0.58 of an inch of rain fell, although the normal rainfall for the period is from 4 to 5 inches. The first samples were taken when the blossoms were forming on the plants, the second as the plants began to set tubers, and the third, when the plants were in the last stages of maturity.

An attempt was made to take samples only on a clear day and they were never taken unless most of the day previous had been clear. This precaution was taken in order that the normal production and utilization of carbohydrates would be occurring within the plant at the time samples were taken. The data in table 1, from the United States Weather Bureau at East Lansing, give the meteorological conditions on the dates of and for two days previous to each sampling.

Regardless of the dry weather, the plants appeared perfectly normal when the first and second samples were taken, although at the time of the first sampling, the plants in the fertilized plots were much larger than those in the check rows. This difference was not so noticeable when the second samples were taken, showing that the larger plants were not growing so rapidly as the smaller ones. On September 4, when the third samples were taken, the plants showed clearly that they were injured by the prolonged drought. The leaves were curled, and the plants looked slightly wilted from the middle of the day until after nightfall, although they were turgid in the morning. No differences could be seen between the size of plants in the check rows and the plants on the fertilized plots.

TABLE 1

Meteorological conditions on sampling dates and two days previous to each sampling

DATE	MINIMUM TEMPERATURE	MAXIMUM TEMPERATURE	CHARACTER OF DAY	SUNSHINE	PRECIPITATION
	°F.	*°F.*		*per cent*	*inches*
July 21	67	89	Partly cloudy	68	0.04
July 22	63	82	Partly cloudy	72	0
July 23	58	83	Clear	99	0
August 10	52	73	Clear	96	0
August 11	45	73	Clear	94	0
August 12	45	73	Clear	100	0
September 2	68	87	Cloudy	23	0.07
September 3	54	82	Clear	95	0
September 4	46	79	Clear	100	0

METHODS OF SAMPLING

At each date of sampling, seven sets of samples were taken. Starting at midnight, samplings were made every four hours until midnight of the following day. In taking the samples, the plants from the whole hill were selected, and the tops cut off about 2 inches above the ground. A sufficient number of hills were taken to fill a 20-pound capacity paper bag with the plants. In the first sampling, the tops from six hills were taken from each fertilized plot, but the plants from eight hills were required from the check plot to give sufficient material for study. Plants from four hills of each fertilized plot and five hills of the check plot gave sufficient material in the second sampling, whereas the plants from four hills, irrespective of treatment, gave sufficient material in the third sampling.

PREPARATION OF SAMPLES

The samples were rushed to the laboratory immediately after cutting, and were separated into two portions—leaves and stalks. A part of each portion was then chopped to pieces with a paper cutter and 50 gm. of each portion was put into separate pint Mason jars, each containing 400 cc. of boiling alcohol to which had been added a little calcium carbonate to neutralize any acids that might be present in the plants. The jars were immediately set on the steam bath to boil for 1 hour in order to kill the enzymes, and thus prevent changes in the carbohydrates present in the plant tissue. The remainder of each sample of leaves and stalks was ground separately in a food chopper and from 100 gm. of the ground tissue the juice was expressed according to the method described by Sayre and Morris (29). The amount of expressed juice from the leaf samples did not in any one case exceed 50 cc. Each lot was measured, however, and poured into bottles containing 200 cc. of boiling alcohol which contained small quantities of calcium carbonate to keep the juice alkaline. The juice from 100 gm. of ground stalks usually amounted to about 60 or 70 cc., 50 cc. of which were pipetted off and preserved in the same manner as the juice from the leaf samples. The samples thus obtained were also put on the steam bath and boiled for one hour. All of the samples of both tissue and juice were tightly sealed in the containers after boiling, and stored to be analyzed later.

METHODS OF ANALYSIS

The samples that were preserved in alcohol were filtered, washed with 80 per cent alcohol, and made up to a volume of 1,000 cc. for tissue samples, and 200 cc. for juice samples. From these quantities aliquot portions were taken and alcohol was removed by distillation under reduced pressure, Claissen flasks being used. The solutions, when reduced to about 30 cc. were washed from the Claissen flasks into 100-cc. volumetric flasks with hot water and cooled down rapidly to room temperature. They were then made up to volume, mixed well, and the contents poured into dry 100-cc. centrifuge tubes containing 0.5 gm. of neutral lead acetate. The solutions thus treated were allowed to stand for 15 or 20 minutes to permit all of the colloidal material to be precipitated, after which they were centrifuged. The supernatant liquids were poured off into other dry centrifuge tubes and powdered sodium oxalate was added to precipitate all of the lead. A second centrifuging settled the lead oxalate precipitates, and the supernatant liquids were decanted into dry beakers. Aliquot parts of these were made up to 100 cc. volume, the size of the aliquot parts depending upon the amount of sugar in the samples.

The solutions thus prepared were thoroughly mixed, 50 cc. of each put into 300-cc. Erlenmeyer flasks for the reducing-sugar determinations, and the remaining 50-cc. portions put into another set of Erlenmeyer flasks containing 5 gm. each of citric acid crystals to hydrolyze the sucrose. The flasks containing the samples for the sucrose determinations were covered with beakers, put on a gas hot plate, and boiled from 10 to 15 minutes to complete the hydrolysis. The citric acid was then neutralized with concentrated sodium hydroxide, phenolphthalein being used as the indicator.

The reducing power of the glucose and hydrolyzed non-reducing sugars was determined by the Shaffer and Hartman (30) method, a standard solution of sodium thio-sulfate being used to titrate the excess iodine set free from a known amount of standard potassium iodide-iodate solution which was added to the reduced copper solutions. Immediately after the iodide-iodate solution was added, 12 cc. of 8 *N* sulfuric acid was added to the solution, the flask shaken to dissolve the cuprous oxide completely, then 20 cc. of a saturated potassium oxalate solution was added and the contents of the flask were titrated with standard sodium thiosulfate solution. When the end-point of the titration was nearly reached, 5 cc. of starch solution was introduced as an indicator and the titration completed.

Because of the buffer effect of the citrate ion in the sucrose samples, it was necessary to add an additional 5 cc. of sulfuric acid in the titration to bring the samples to the necessary pH.

The committee on methods for the Society of Plant Physiology (19) objects to the use of the Shaffer and Hartman method on the ground that for various plant tissues, it is not always possible to obtain a sharp end-point in the final titration. In this work, by using the recommended 15 cc. of 5 *N* sulfuric acid, the end-point was not always sharply defined, but by increasing the strength of the acid, a clearly defined end-point could always be obtained. It appears, at least with the plant tissue used in this work, that there is a rather narrow range of pH in which a sharp end-point can be obtained when this method is used. If an excess of acid is added, a white precipitate forms in the solution and the resulting titration values are too low, whereas if the pH is too high, the end-point is indistinct and the results are unreliable. The satisfactory titration after the addition of extra acid, in the case of sucrose hydrolyzed by citric acid, to correct for the buffer effect of the citrate ion, indicates the necessity of having the proper degree of acidity in the solutions. With a little practice, one can usually tell by the color of the solutions as the acid is being added just how much to use, and if one is working with tissue from only one kind of plant, the correct acidity can soon be found and no further difficulty will be encountered.

If the difficulty lies in obtaining a definite pH value in the solutions before titrating, it is clear why the tissue of different plants cannot be handled alike, because different tissues may exert a different buffer effect on the solutions, which would necessarily have to be corrected before sharp end-points could be obtained.

There are a number of other methods of determining the amount of reduced copper (11, 19), but the Shaffer and Hartman method has a distinct advantage over most of them in that it is not necessary in this method to filter off the products of the reaction before the titration can be made for the determination of reduced copper. An excellent review of the literature on methods is given in the latter reference.

Although the method of reducing the copper described by Quisumbing and Thomas (26) was employed in this work, because it was more convenient than the Munson and Walker method (22) in handling a number of samples at one time, the Munson and Walker tables were employed for calculating the amount

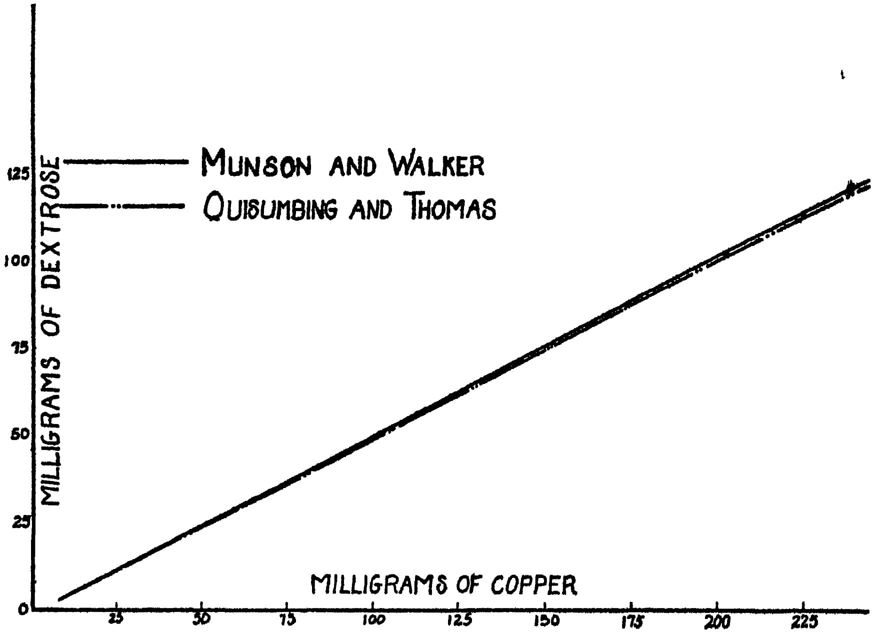

FIG. 1. A COMPARISON OF THE MUNSON AND WALKER AND THE QUISUMBING AND THOMAS TABLES FOR CALCULATING THE QUANTITY OF SUGAR FROM THE AMOUNT OF COPPER REDUCED

of sugar from the amount of copper reduced, because they are much more inclusive. The curves in figure 1 show the variations between the two tables within the range of copper used in this work. The difference between the two is so slight that the error due to interpolation in using the less complete tables of Quisumbing and Thomas would be greater in many cases than the difference between the two tables.

It was desirable to have a plan whereby the set of eight samples at each cutting, could be analyzed in one day in order to treat all samples as nearly alike as possible even with respect to the time between starting a determination and the final titration. In order to attain this end, it was necessary to use certain procedures, such as distillation under reduced pressure to remove the excess alcohol, and the use of the citric acid hydrolysis for sucrose determinations, because of their greater speed and adaptation to the scheme of analysis. Using the centrifuge to settle precipitates is much more rapid and convenient than filtering. Careful tests of these procedures showed that accuracy was not sacrificed for speed.

EXPERIMENTAL RESULTS

The data presented in this paper are all expressed in per cent based on the green weight. All free-reducing sugars are reported as glucose and all material hydrolyzed with 10 per cent citric acid as sucrose. Total sugars are the sum of the two.

The diurnal variations of the sugars for the first sampling are shown in figures 2 and 3. The curves for the various treatments do not parallel one another as well in figure 2 as they do in figure 3. This shows that there were more variations in the glucose than in the sucrose throughout the day. Glucose variations showed rather well-defined minimums at 4 a.m. The maximums are not so marked but in general they occurred either at noon or 4 p.m. Sucrose, on the other hand, seemed to maintain a more constant level, showing neither a well-defined minimum or maximum. In practically all cases, the amounts of glucose were in excess of sucrose.

Figures giving diurnal variations for the second and third samplings are omitted because in general they are similar to figures 2 and 3. The percentages of glucose and sucrose are both somewhat higher in the later samplings, and the curves are a little more irregular.

The mode in which sucrose and glucose concentrations varied throughout the day, as reported in this paper, supports the work of Davis and Sawyer (9), who studied carbohydrate variations in the leaves and stalks of the potato plants, and Miller (21), who investigated the carbohydrate relations in the leaves of corn and sorghums. These investigators found glucose to have a minimum early in the day and a maximum in the early afternoon, whereas sucrose had a tendency to maintain an almost constant level throughout the day. They found sucrose, however, to be in excess of glucose in practically all cases, whereas the data herewith reported, with few exceptions, showed glucose to be higher than sucrose. The results reported in this work are in accord with those of Clements (7), who found that glucose was much higher than sucrose in the leaves of potatoes, sunflowers, and soybeans for the early stages of growth. Throughout the later periods of growth, the differences noted by him were not so great, but in general, the amount of glucose was in excess.

The averages of the seven samples taken at 4-hour intervals on each date of sampling, are given as daily averages in tables 2, 3, and 4. Table 2 is arranged to show the effect of fertilizer treatment on the concentrations of glucose and sucrose in the various parts of the plant.

A comparison of the averages (shown as the sum of the averages in table 2) for all samplings of leaf tissue, points out that the 0–16–8 treatment gave the lowest concentrations of sugars, whereas the check and the 4–16–8 gave the highest. In the leaf sap, the 0–16–8 continued to average lowest, with the 4–16–8 highest and the check and 4–16–0 intermediate. In the stalk tissue the check showed the highest average, with 4–16–0 and 4–16–8 about equal to each other and about midway between the check and 0–16–8 averages. In the stalk sap the check and 4–16–0 were considerably higher than the other two with the 0–16–8 still averaging lowest.

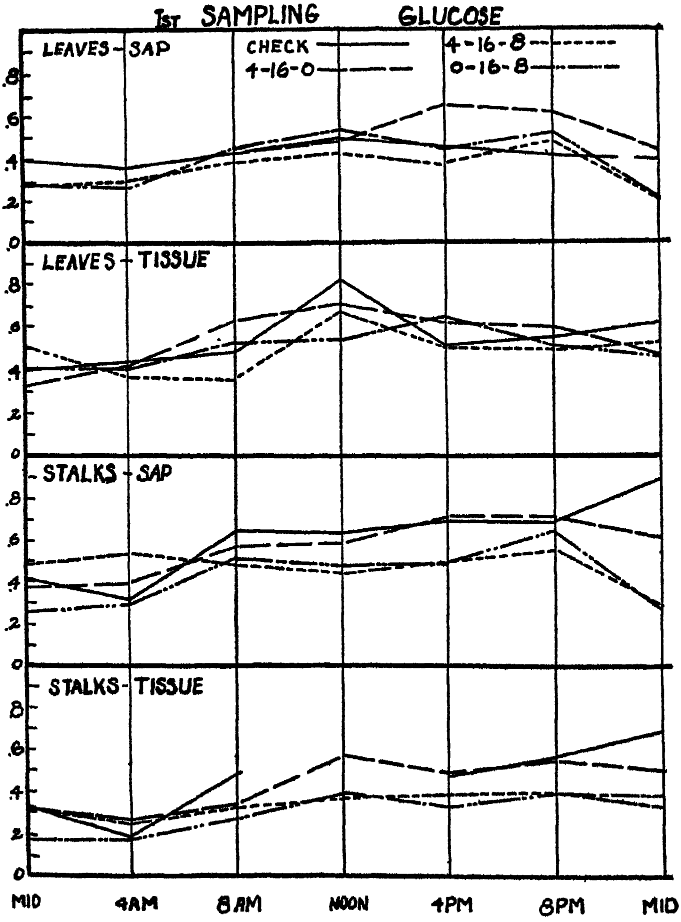

Fig. 2. Results of Fertilizer Treatment on Per Cent of Glucose in Samples Taken July 23, 1930

The general summary, shown as the mean in the last line running from left to right in table 2, gives the average of the sum of averages. It points out that for both tissue and sap, the check has the highest average sugar content and the 0–16–8 the lowest, with 4–16–0 and 4–16–8 about equal to each other and about midway between the extremes. These differences are not significant, showing that there were no differences in sugar content attributable to fertilizer treatments. A comparison of the daily averages for each sampling (table 2) indicates that the relation of the different treatments as shown by the mean was seldom the same as that found in any sampling. The same relation did not exist for the various treatments between determinations made from tissue and sap from the same plants, yet a comparison of the mean showed them to be very similar. Janssen and Bartholomew (16), working with plants in the field and in sand and water cultures, found no differences in carbohydrate content in plants grown in the field under varied potash treatments. When the same plants were grown in sand and water cultures, they found that concentrations of 3 p.p.m. gave maximum carbohydrate production, indicating

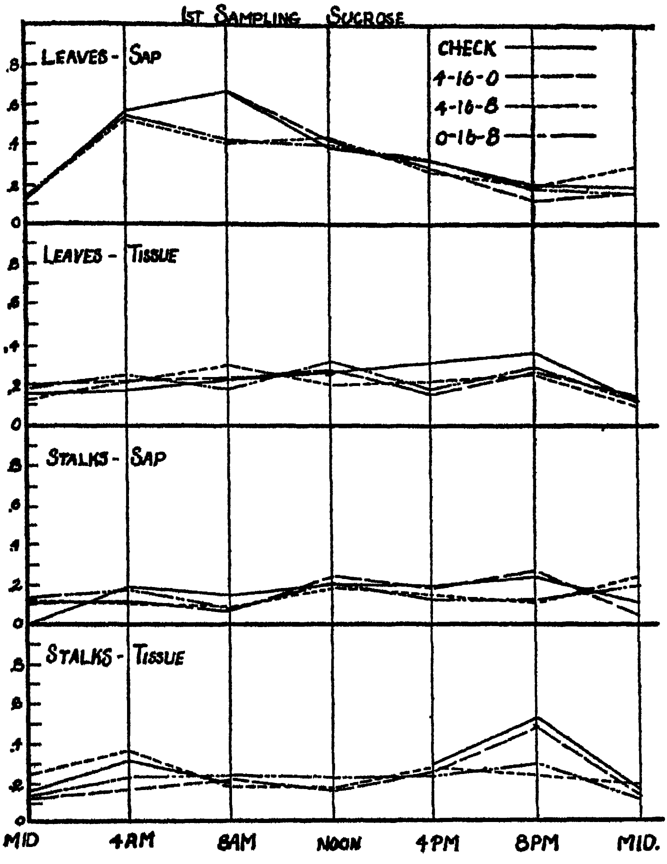

FIG. 3. RESULTS OF FERTILIZER TREATMENT ON PER CENT OF SUCROSE IN SAMPLES TAKEN JULY 23, 1930

that under field conditions, because of so many variables, the effect of fertilizer treatments is not manifest in variations of carbohydrate content, although the treatments may greatly increase the yields.

TABLE 2

Sugars in potato plants (daily averages) as per cent of green weights

SAMPLING PERIOD	WHOLE TISSUE				EXPRESSED SAP			
	Fertilizer treatments							
	Check	4-16-0	0-16-8	4-16-8	Check	4-16-0	0-16-8	4-16-8
	Glucose—leaves				*Glucose—leaves*			
First	0.545	0.541	0.492	0.483	0.412	0.479	0.384	0.342
Second	0.606	0.624	0.582	0.634	0.535	0.512	0.583	0.600
Third	0.722	0.689	0.675	0.804	0.492	0.523	0.469	0.498
Average	0.624	0.618	0.583	0.640	0.480	0.505	0.479	0.480
	Sucrose—leaves				*Sucrose—leaves*			
First	0.233	0.217	0.213	0.202	0.348	0.293	0.305	0.324
Second	0.431	0.421	0.403	0.461	0.416	0.395	0.290	0.426
Third	0.525	0.393	0.447	0.462	0.474	0.503	0.540	0.601
Average	0.396	0.344	0.354	0.375	0.413	0.397	0.378	0.450
Sum of averages	1.020	0.962	0.937	1.015	0.893	0.902	0.857	0.930
	Glucose—stalks				*Glucose—stalks*			
First	0.456	0.434	0.305	0.343	0.613	0.563	0.419	0.467
Second	0.719	0.671	0.530	0.483	0.786	0.788	0.697	0.649
Third	0.936	0.898	0.798	0.957	0.852	0.839	0.653	0.741
Average	0.704	0.668	0.544	0.594	0.750	0.730	0.590	0.619
	Sucrose—stalks				*Sucrose—stalks*			
First	0.237	0.219	0.209	0.236	0.154	0.145	0.145	0.145
Second	0.368	0.310	0.327	0.313	0.199	0.153	0.214	0.221
Third	0.378	0.335	0.379	0.371	0.378	0.414	0.379	0.451
Average	0.328	0.288	0.305	0.307	0.244	0.237	0.246	0.272
Sum of averages	1.032	0.956	0.849	0.901	0.994	0.967	0.836	0.891
Mean	1.026	0.959	0.893	0.958	0.944	0.935	0.847	0.911

The $\frac{\text{glucose}}{\text{sucrose}}$ ratio has often been used as a means of showing carbohydrate metabolism in plants. Table 3 shows the daily averages of glucose and sucrose for the various treatments at the different periods of sampling and gives the $\frac{\text{glucose}}{\text{sucrose}}$ ratio for each. For some of the periods of sampling the ratio was quite constant for all treatments, whereas with others, there were wide variations. The ratio for the second sampling in the leaf tissue was very uniform and with one exception, was very uniform in the leaf sap. In the sap of both leaves and stalks for the third sampling, the ratio was lower for the 0–16–8 and 4–16–8 than the other two treatments. In the remaining instances the ratio

TABLE 3

Comparison of glucose and sucrose from same samples (daily averages)

FERTILIZER TREATMENT	FIRST SAMPLING			SECOND SAMPLING			THIRD SAMPLING			MEAN OF RATIO
	Glucose	Sucrose	$\frac{\text{Glucose}}{\text{Sucrose}}$	Glucose	Sucrose	$\frac{\text{Glucose}}{\text{Sucrose}}$	Glucose	Sucrose	$\frac{\text{Glucose}}{\text{Sucrose}}$	
Leaves—tissue										
Check	0.545	0.233	1.91	0.606	0.431	1.41	0.722	0.525	1.37	1.56
4–16–0	0.541	0.217	2.49	0.624	0.421	1.48	0.689	0.393	1.72	1.90
0–16–8	0.492	0.213	2.31	0.582	0.403	1.44	0.675	0.447	1.51	1.75
4–16–8	0.483	0.202	2.39	0.634	0.461	1.38	0.804	0.462	1.73	1.83
Average...	0.515	0.216	2.27	0.611	0.429	1.43	0.722	0.457	1.58	1.75
Leaves—sap										
Check	0.412	0.348	1.18	0.535	0.416	1.28	0.492	0.474	1.04	1.17
4–16–0	0.479	0.293	1.63	0.512	0.395	1.30	0.523	0.503	1.04	1.32
0–16–8	0.384	0.305	1.26	0.583	0.290	2.01	0.469	0.540	0.87	1.38
4–16–8	0.342	0.324	1.06	0.600	0.426	1.41	0.498	0.601	0.83	1.10
Average...	0.404	0.317	1.28	0.557	0.382	1.50	0.495	0.529	0.94	1.24
Stalks—tissue										
Check	0.456	0.237	1.92	0.719	0.368	1.95	0.936	0.378	2.47	2.17
4–16–0	0.434	0.219	1.98	0.671	0.310	2.16	0.898	0.335	2.68	2.27
0–16–8	0.305	0.209	1.44	0.530	0.327	1.62	0.798	0.379	2.11	1.72
4–16–8	0.343	0.236	1.45	0.483	0.313	1.54	0.957	0.371	2.58	1.86
Average...	0.384	0.225	1.69	0.601	0.329	1.82	0.897	0.366	2.46	2.00
Stalks—sap										
Check	0.613	0.154	4.00	0.786	0.199	3.95	0.852	0.378	2.25	3.40
4–16–0	0.563	0.145	3.88	0.788	0.153	5.15	0.839	0.414	2.02	3.68
0–16–8	0.419	0.145	2.89	0.697	0.214	3.26	0.653	0.379	1.72	2.62
4–16–8	0.467	0.145	3.22	0.649	0.221	2.94	0.741	0.451	1.64	2.60
Average...	0.515	0.147	3.49	0.730	0.197	3.82	0.771	0.405	1.91	3.10

for the various treatments was very variable but there seemed to be no definite treatment that was outstandingly high or low.

The average $\frac{\text{glucose}}{\text{sucrose}}$ ratio for all four treatments was higher in the leaf

TABLE 4

Comparison of the sugars in leaves and stalks of potato plants (daily averages)

TREATMENT	FIRST SAMPLING			SECOND SAMPLING			THIRD SAMPLING			MEAN OF $\frac{\text{LEAVES}}{\text{STALKS}}$
	Leaves	Stalks	$\frac{\text{Leaves}}{\text{Stalks}}$	Leaves	Stalks	$\frac{\text{Leaves}}{\text{Stalks}}$	Leaves	Stalks	$\frac{\text{Leaves}}{\text{Stalks}}$	
					Tissue–glucose					
Check	0.545	0.456	1.19	0.606	0.719	0.84	0.722	0.936	0.77	0.93
4–16–0	0.541	0.434	1.24	0.624	0.671	0.93	0.689	0.898	0.77	0.98
0–16–8	0.492	0.305	1.61	0.582	0.530	1.10	0.675	0.798	0.84	1.18
4–16–8	0.483	0.343	1.41	0.634	0.483	1.31	0.804	0.957	0.84	1.19
Average...	0.515	0.384	1.36	0.611	0.601	1.04	0.722	0.897	0.80	1.07
					Tissue—sucrose					
Check	0.233	0.237	0.99	0.431	0.368	1.17	0.525	0.378	1.39	1.18
4–16–0	0.217	0.219	1.00	0.421	0.310	1.32	0.393	0.335	1.18	1.17
0–16–8	0.213	0.209	1.01	0.403	0.327	1.23	0.447	0.379	1.18	1.18
4–16–8	0.202	0.236	0.86	0.461	0.313	1.47	0.462	0.371	1.24	1.19
Average...	0.216	0.225	0.97	0.429	0.329	1.29	0.457	0.366	1.25	1.17
					Sap—glucose					
Check	0.412	0.613	0.67	0.535	0.786	0.68	0.492	0.852	0.58	0.64
4–16–0	0.479	0.563	0.85	0.512	0.788	0.65	0.523	0.839	0.62	0.71
0–16–8	0.384	0.419	0.92	0.583	0.697	0.83	0.469	0.653	0.72	0.84
4–16–8	0.342	0.467	0.73	0.600	0.649	0.93	0.498	0.741	0.67	0.78
Average...	0.404	0.515	0.79	0.557	0.730	0.77	0.495	0.771	0.65	0.74
					Sap—sucrose					
Check	0.348	0.154	2.26	0.416	0.199	2.09	0.474	0.378	1.25	1.87
4–16–0	0.293	0.145	2.02	0.395	0.153	2.58	0.503	0.414	1.22	1.94
0–16–8	0.305	0.145	2.10	0.290	0.214	1.35	0.540	0.379	1.42	1.62
4–16–8	0.324	0.145	2.23	0.426	0.221	1.93	0.601	0.451	1.33	1.82
Average...	0.317	0.147	2.15	0.382	0.197	1.99	0.529	0.405	1.30	1.81

tissue in the early stages of growth than in the later, whereas in the stalk tissue, the ratio increased as the plants matured. On the other hand, for the sap the ratio was highest in the second sampling, and lowest in the third for both leaves and stalks.

The mean of the $\frac{\text{glucose}}{\text{sucrose}}$ ratio for all three samplings is shown in the column at the extreme right in table 3. This mean ratio showed the 4–16–0 to have the highest ratio in three of the four instances, whereas each of the other three treatments is lowest once and the 0–16–8 and 4–16–8 showed the lowest average in the fourth case. The ratio is somewhat lower for the leaves than for the stalks. In general, the $\frac{\text{glucose}}{\text{sucrose}}$ ratio of the daily averages showed that no treatment is significantly high or low.

In order to determine whether there was any difference in the rate of translocation of carbohydrates, the concentrations of sugars in the leaves are compared with those of the stalks. The comparisons are made on the daily averages and expressed as the $\frac{\text{leaves}}{\text{stalks}}$ ratio, as shown in table 4. In general, in the tissue there was more glucose in the leaves than in the stalks of the first sampling, and less in the third, whereas in the second the check and 4–16–0 had less and the 0–16–8 and 4–16–8 had more glucose in the leaves than in the stalks. The average of the ratios for all three periods decreased with the age of the plants. The mean of the ratios of all three periods showed the check and 4–16–0 to be just slightly less than unity with the other two treatments considerably higher.

The ratios in the sap are not the same as those of the tissue. For glycose, the average of all treatments showed a ratio of less than 1 for all three samplings, whereas the ratio for sucrose is greater than 1. For both glucose and sucrose the ratio is highest in the first sampling and lowest in the third.

DISCUSSION

From the experimental results presented, no differences in sugar content, attributable to variations in fertilizer treatment were obtained, and yet rather wide differences in the per cent composition at any one time, as shown in figure 2, were often observed. Similar irregularities are reported by Clements, Miller and others. There seem to be three possible reasons for such inconsistent variations.

First, the carbohydrate content is much more affected by other factors not under control in this experiment than by variations in fertilizer treatment. This possibility is supported by the work of Arthur, Guthrie, and Newell (1), who grew a number of plants under very carefully controlled conditions, such as control of temperature, humidity, moisture, light, and plant nutrients. They found that the intensity of, and time of exposure, to light were much more important factors in carbohydrate formation than were the fertilizers which were used. In this present work, no attempt was made to measure the intensity and time of exposure to light on the various plots, which were all located within a radius of 4 to 5 rods. It was assumed that for such a small area there would be no variation in atmospheric conditions of sufficient magnitude to bring about any differences in sugar content of the plants.

Second, the method of analysis is not sufficiently accurate to give consistent results. This possibility seems rather remote because very careful tests were made on the alcoholic extracts of both tissue and sap as well as on pure solutions of glucose and sucrose by a number of methods and in all cases accurate checks were obtained.

Third, more plausible explanation than the other two, the analysis of the reducing power does not give the true relation of the various sugars as they existed in the plants at the time of sampling. Webster (31) found that the nitrate nitrogen content of alcoholic extracts decreases upon standing, and suggested that sugars might vary in a like manner. Results obtained in preliminary studies to this work, however, indicated that the composition of glucose and sucrose does not change in alcoholic extracts upon standing for at least 6 months. The change appears to have taken place between the time the samples were taken in the field and the time when all enzymes had been killed by boiling in alcohol for 1 hour. Before accurate results can be obtained in this type of investigation, it is necessary to perfect a method of sampling and preparing fresh plant material for analysis that will assure the investigator that the material he analyzes is really showing the carbohydrate relations as they existed in the plant at the time the samples were taken.

SUMMARY

Analyses showing diurnal and seasonal variations in the sugar content of both the sap and tissue of the potato plant are presented.

The percentage of glucose is in excess of sucrose in practically all samples of both leaves and stalks.

The diurnal variation of glucose showed a more or less definite cycle, with minimum concentrations usually occurring at 4 or 8 a.m., and the maximums either at noon or 4 p.m., whereas sucrose remained about constant throughout the day.

The amounts of total sugar ran higher as the plants got older.

Under the conditions of this experiment, no differences were seen in sugar content attributable to the various fertilizer treatments.

An opinion as to why carbohydrate analyses show such irregularity of variation in this and other work is presented.

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THE USE OF THE MOISTURE EQUIVALENT IN THE TEXTURAL CLASSIFICATION OF SOILS

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Some form of mechanical analysis has been used in this country for many years as a means for assigning textural class names to soils encountered during soil surveys. The function of a textural name is considered to be that of conveying an approximate idea of the size distribution curve of a soil. In this case it is apparent that the mechanical analysis, if such can be performed in a manner providing results of fundamental significance, is the most reasonable basis for such a classification. When used in this capacity the individual soil samples dealt with are usually part of a succession of depth samples collected from a single soil profile which is considered to be representative, as judged by field observation, of the entire area covered by a single soil type.

In addition, the textural name is widely used by laboratory and field workers to convey some idea of the field character and behavior of the soil. Any method of mechanical analysis which aims at complete dispersion necessarily includes in its early stages the partial or entire reconstruction of the system colloid-absorbed cations. However desirable this may be from the viewpoint of the investigator in other respects, it gives a more or less imperfect picture of the physical properties of the soil as it exists in the field. The ideas conveyed by this statement have been dealt with by Keen (9, 10) and will not be further enlarged upon. Although the mechanical analysis of a soil sample after a high degree of dispersion may be regarded at present as the best, and the least arbitrary, reference point for the expression of its texture, and although it constitutes a valuable method of approach for certain kinds of research in soil physics, it is well known that a gap exists between the mechanical analysis and its interpretation in terms of field behavior. Zunker (18, 19) has made extensive observations in this direction, having examined the relations existing between the specific surface and certain field characteristics of soils.

A question arises, therefore, concerning the advisability of making expensive analyses of single soil samples, which at best are slightly variable from place to place, for which the immediate need is some basis for textural classification and an indication of probable field behavior. Various single-valued expressions have been used for the purpose of reducing the time involved in mechanical analyses and the unwieldiness of the results obtained. No application of any single-valued expression to textural classification appears to have been made, however, although various equations have been proposed to express the approximate relation existing between certain of these and the corresponding mechanical analyses of soil samples.

The moisture equivalent was originally proposed by Briggs and McLane in 1907 as a purely arbitrary single-valued expression of water retentive power and was later mentioned by them as a possible basis for soil classification (4). The connections between moisture equivalent and water retaining capacity of soil columns both in field and laboratory have since received much attention, and useful relationships have been established (2, 7, 13, 17). For this reason the moisture equivalent has much interest under the agricultural conditions of western North America and wherever moisture may be the limiting factor in production. It has also been shown to bear a general, though not exact, relation to the permanent wilting percentage of soils (5, 1, 16) and to the non-sticky plastic range (3).

The following equations have been proposed to connect the mechanical analysis of a soil and its moisture equivalent:

Briggs and Shantz, 1912 (4):
$$\text{M.E.} = 0.02 \text{ sand} + 0.22 \text{ silt} + 1.05 \text{ clay}$$
Alway and Russel, 1916 (1):
$$\text{M.E.} = 0.14 \text{ sand} + 0.27 \text{ silt} + 0.53 \text{ clay}$$
Smith, 1917 (14):
$$\text{M.E.} = 0.023 \text{ sand} + 0.25 \text{ silt} + 0.61 \text{ clay}$$
Middleton, 1920 (11):
$$\text{M.E.} = 0.063 \text{ sand} + 0.291 \text{ silt} + 0.42 \text{ clay}$$

Smith's equation is the result of direct determinations of the moisture equivalents of soil separates obtained from several Californian soil types of varying texture. The others were calculated by the method of least squares. In all cases the terms "sand," "silt," and "clay" refer to the U. S. Bureau of Chemistry and Soils classification for analyses made by the old centrifugal method following simple pretreatment by shaking with ammonia water only. It is uncertain to what extent these equations would be changed if the mechanical analyses were to be made by methods producing a higher state of dispersion than was obtained by the ammonia pretreatment and centrifugal separation. In a large number of cases the clay content might be expected to be increased at the expense of the silt, thus changing the relative weights attributable to these two components in the calculation of the moisture equivalent. Thomas and Harris (15) and Joseph (8) have examined the effect of the constitution of the clay and silt particles upon the moisture equivalent. It is evident from their results that the chemical composition of the particle itself and of its absorbed cations may influence the moisture equivalent value considerably.

The additive nature of the effect upon the moisture equivalent of the physical components of the soil has been investigated further in this laboratory by the direct method of measuring the moisture equivalents of mixtures of soil of known moisture equivalent values. The results so obtained led to a consideration of the utility of a combined moisture equivalent-soil class diagram and the application of the moisture equivalent for purposes of textural classification. The method and results follow.

MOISTURE EQUIVALENTS OF SOIL MIXTURES

Procedure

Five samples of soil and a quartz sand were used in the experiments. Both soils and sand were air-dry. The finer materials contained many hard lumps which necessitated the use of a rubber-tipped pestle and mortar, and in the case of the Sacramento clay a hard-tipped pestle, to reduce them to a condition fine enough to pass through a 1-mm. brass sieve. A 1-mm. rather than a 2-mm. sieve was used to make possible the preparation of more nearly homogeneous mixtures of soil. The samples were thoroughly mixed, placed in glass jars, and sealed, after which their contents of moisture were determined. From the moisture content of each a table was prepared, giving the different weights of each air-dry sample which it would be necessary to take in preparing a series of mixtures with a second sample in which the two samples would be present in certain proportions on the dry basis. For every pair of the six samples, seven mixtures were prepared, such that one member of every pair was present as 12 5, 25, 37.5, 50, 62.5, 75, and 87.5 per cent, dry basis, of the mixture. Sufficient material was taken in the preparation of each mixture to give 90 gm. of mixture, the mixing being done as thoroughly as possible on a sheet of paper with a spatula. The six samples provided 15 possible combinations, each combination being used in the seven different proportions already mentioned. The moisture equivalent determinations of the original samples and of their mixtures were made in duplicate by the usual method of procedure used at this station, the speed of the centrifuge being checked by four speed measurements made with a Peerboom and Schurmann tachometer at 10-minute intervals during each run. The moisture equivalents were expressed as percentages of the dry weight of the material. In case of deviations between duplicates in excess of 2 per cent of their average moisture equivalent, the

TABLE 1

Moisture equivalents of the soils used in preparation of the mixtures

NUMBER	NAME	MOISTURE EQUIVALENT
		per cent
24	Quartz sand	0 34
13	Gravelly sand	4 4
14	Yolo sandy loam	14 3
15	Yolo sandy loam	21 1
22	Yolo loam	24 4
30	Sacramento clay	36 2

TABLE 2

Moisture equivalents of soil mixtures

COMBINATION NO.	SOILS USED IN COMBINATION*		*m*		PERCENTAGE (*x*) OF SOIL *b* PRESENT IN MIXTURE									AVERAGE DEVIATION
	Soil *a*	Soil *b*			0	12.5	25.0	37.6	50.0	62.5	75.0	87.5	100	
					per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	
A	15	22	0.033	Calculated M.E.†	*21.1*	*21.5*	*21.9*	*22.3*	*22.8*	*23.2*	*23.6*	*24.0*	*24.4*	
				Observed M.E.	21.1	21.8	21.9	22.4	22.7	23.0	23.9	24.2	24.4	
				Difference	....	+0.3	0	+0.1	−0.1	−0.2	+0.3	+0.2	....	±0.17
B	24	13	0.041	Calculated M.E.	*0.3*	*0.8*	*1.3*	*1.8*	*2.4*	*2.9*	*3.4*	*3.9*	*4.4*	
				Observed M.E.	0.3	1.0	1.0	1.8	2.4	2.8	3.7	3.9	4.4	
				Difference	....	+0.2	−0.3	0	0	−0.1	+0.3	0	....	±0.13
C	14	15	0.068	Calculated M.E.	*14.3*	*15.2*	*16.0*	*16.9*	*17.7*	*18.6*	*19.4*	*20.4*	*21.1*	
				Observed M.E.	14.3	14.9	16.6	17.5	17.9	18.7	19.6	19.2	21.1	
				Difference	....	−0.3	+0.6	+0.6	+0.2	+0.1	+0.2	−1.2	....	±0.46
D	13	14	0.099	Calculated M.E.	*4.4*	*5.6*	*6.9*	*8.1*	*9.4*	*10.6*	*11.8*	*13.1*	*14.3*	
				Observed M.E.	4.4	5.7	7.0	8.3	9.4	11.2	11.3	13.2	14.3	
				Difference	....	+0.1	+0.1	+0.2	0	+0.6	−0.5	+0.1	....	±0.23
E	14	22	0.101	Calculated M.E.	*14.3*	*15.6*	*16.8*	*18.1*	*19.3*	*20.6*	*21.9*	*23.1*	*24.4*	
				Observed M.E.	14.3	15.8	17.3	18.2	20.0	21.6	21.9	23.4	24.4	
				Difference	....	+0.2	+0.5	+0.1	+0.7	+1.0	0	+0.3	....	±0.40
F	22	30	0.118	Calculated M.E.	*24.4*	*25.9*	*27.3*	*28.8*	*30.3*	*31.8*	*33.3*	*34.7*	*36.2*	
				Observed M.E.	24.4	25.0	26.9	28.0	29.1	31.0	33.3	34.9	36.2	
				Difference	....	−0.9	−0.4	−0.8	−1.2	−0.8	0	+0.2	....	±0.61
G	24	14	0.140	Calculated M.E.	*0.3*	*2.1*	*3.8*	*5.5*	*7.3*	*9.0*	*10.8*	*12.6*	*14.3*	
				Observed M.E.	0.3	—	4.6	5.8	7.5	9.4	10.8	12.6	14.3	
				Difference	....	—	+0.8	+0.3	+0.2	+0.4	0	0	....	±0.28

H	15	30	0.151	Calculated M.E.	*21.1*	*23.0*	*24.9*	*26 8*	*28.6*	*30.5*	*32.4*	*34.3*	*36.2*	
				Observed M.E.	21.1	23.7	25.3	26.5	28.1	29.7	32.3	34.8	36.2	
				Difference	....	+0.7	+0.4	−0.3	−0.5	−0.8	−0.1	+0.5	....	±0.47
I	13	15	0.167	Calculated M.E.	*4.4*	*6.5*	*8.6*	*10.7*	*12.8*	*14.8*	*16.9*	*19.0*	*21.1*	
				Observed M.E.	4.4	6.6	9.1	11.6	13.5	15.7	17.3	18.7	21.1	
				Difference	....	+0.1	+0.5	+0.9	+0.7	+0.9	+0.4	−0.3	....	±0.54
J	13	22	0.200	Calculated M.E.	*4.4*	*6.9*	*9.4*	*11.9*	*14.4*	*16.9*	*19.4*	*21.9*	*24.4*	
				Observed M.E.	4.4	7.4	10.5	13.3	15.9	18.0	20.4	22.6	24.4	
				Difference	....	+0.5	+1.1	+1.4	+1.5	+1.1	+1.0	+0.7	....	±1.04
K	24	15	0.208	Calculated M.E.	*0.3*	*2.9*	*5.5*	*8.1*	*10.7*	*13.3*	*15.9*	*18.5*	*21.1*	
				Observed M.E.	0.3	3.4	5.3	9.0	11.4	14.3	16.6	19.3	21.1	
				Difference	....	+0.5	−0.2	+0.9	+0.7	+1.0	+0.7	+0.8	....	±0.69
L	14	30	0.219	Calculated M.E.	*14.3*	*17.0*	*19.8*	*22.5*	*25.3*	*28.0*	*30.7*	*33.5*	*36.2*	
				Observed M.E.	14.3	17.1	20.7	23.6	25.2	28.4	31.0	35.2	36.2	
				Difference	....	+0.1	+0.9	+1.1	−0.1	+0.4	+0.3	+1.7	....	±0.66
M	24	22	0.241	Calculated M.E.	*0.3*	*3.3*	*6.3*	*9.3*	*12.4*	*15.4*	*18.4*	*21.4*	*24.4*	
				Observed M.E.	0.3	3.7	7.6	10.7	13.4	17.7	19.3	21.5	24.4	
				Difference	....	+0.4	+1.3	+1.4	+1.0	+2.3	+0.9	+0.1	....	±1.06
N	13	30	0.318	Calculated M.E.	*4.4*	*8.4*	*12.4*	*16.3*	*20.3*	*24.3*	*28.2*	*32.2*	*36.2*	
				Observed M.E.	4.4	9.2	14.0	17.9	21.5	25.3	28.9	34.0	36.2	
				Difference	....	+0.8	+1.6	+1.6	+1.2	+1.0	+0.7	+1.8	....	±1.24
O	24	30	0.359	Calculated M.E.	*0.3*	*4.8*	*9.3*	*13.8*	*18.3*	*22.7*	*27.2*	*31.7*	*36.2*	
				Observed M.E.	0.3	5.4	10.4	15.4	20.0	23.3	28.3	32.8	36.2	
				Difference	....	+0 6	+1.1	+1.6	+1.7	+0.6	+1.1	+1.1	....	±1.11

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* See table 1 for names of soils corresponding to the numbers here given.

† M.E. = Moisture equivalent.

determinations were repeated for those soil mixtures having a moisture equivalent greater than 10 per cent; for the coarser textured soils wider deviations were permitted.

The series and class names of the soils represented by these samples are given in table 1. The class names are obtained from the mechanical analyses previously determined by the old centrifugal method and by reference to the triangular diagram of Davis and Bennett (6). Sample 15 is a heavy sandy loam, almost a loam. Sample 22 is a heavy loam, and as judged by its feel is generally referred to as a clay loam. The mechanical analysis shows it to be on the boundary between a loam and a clay loam.

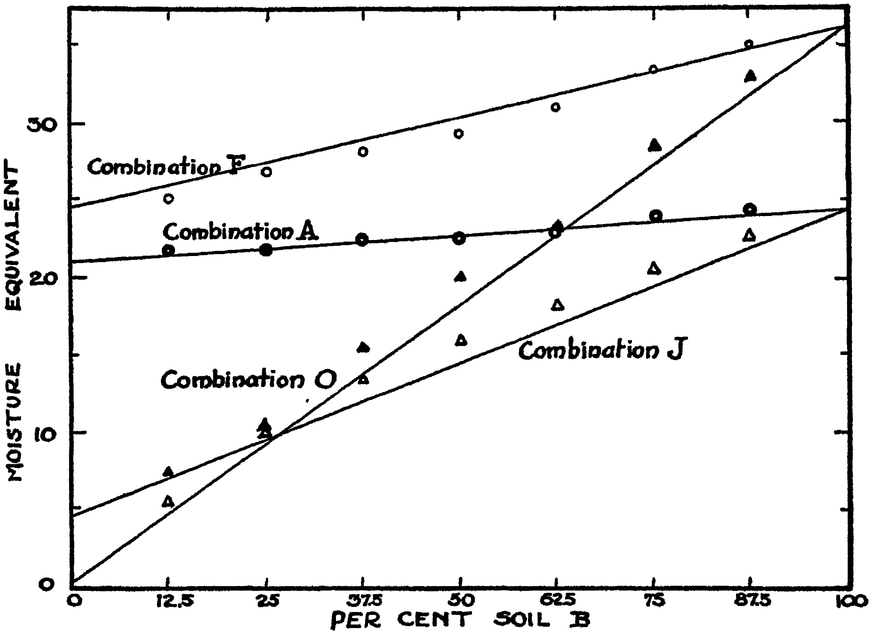

FIG. 1. CALCULATED AND OBSERVED MOISTURE EQUIVALENTS FOR FOUR SOIL COMBINATIONS

Combination A—soil 15 (M.E. = 21.1) and soil 22 (M.E. = 24.4).
Combination F—soil 22 (M.E. = 24.4) and soil 30 (M.E. = 36.2).
Combination J—soil 13 (M.E. = 4.4) and soil 22 (M.E. = 24.4).
Combination O—soil 24 (M.E. = 0.3) and soil 30 (M.E. = 36.2).

Table 2 contains the average results of the duplicate determinations of the moisture equivalents of the samples and their mixtures. That member of a soil combination having the lower moisture equivalent is designated *soil a*, the member having the higher moisture equivalent being termed *soil b*. The calculated and observed moisture equivalents for combinations A, F, J, and O are represented in figure 1. The observed moisture equivalent values evidently lie very close to a straight line connecting the moisture equivalents of the component samples. Assuming an entirely additive relationship between moisture equivalent and physical components, the equation of the line representing the moisture equivalent of mixtures of soil samples of known moisture equivalent values may be written as

$$y = mx + y_a$$

where y = the moisture equivalent in per cent of a homogeneous mixture of two soils, a and b.

y_a = the moisture equivalent of the soil having the lower moisture equivalent.

y_b = the moisture equivalent of the soil having the higher moisture equivalent.

$m = \frac{y_b - y_a}{100}$.

and x = per cent of soil b in the mixture.

The value of y in the foregoing equation has been calculated for each mixture in the 15 combinations and the calculated values are included in table 2, in italics. The soil combinations and the resulting moisture equivalents are tabulated in order of increasing values of m, that is, in order of increasing difference between y_b and y_a. A close parallelism between observed and calculated values is evident throughout.

From the average deviations contained in the righthand column of table 2, it is evident that greater difference between observed and calculated values occurs in mixtures having widely different moisture equivalents. The average deviation for all mixtures is ±0 61 moisture equivalent. For those mixtures in which y_b exceeded y_a by a moisture equivalent percentage of 20 or less the average deviation is only ±0.44. The increase in the deviation which occurs with increase in m is evidently due to the difficulty of obtaining a uniform mixture of the component materials, the difficulty becoming greater with decrease in similarity of proportions of different sized particles. Other errors of operation are probably emphasized in the result when the soil samples concerned are very dissimilar.

These results suggest the direct application of Smith's values connecting the moisture equivalent and mechanical analysis to the soil texture diagram of Davis and Bennett. Suppose the equilateral triangle diagram, with its inscribed class boundaries and triangular coördinate system be considered as the base, ABC, of a truncated prism (see fig. 2), having vertical edges measuring upwards respectively 2.3 units (AA′) from the 100 per cent sand apex, 25 units (BB′) from the 100 per cent silt apex, and 61 units (CC′) from the 100 per cent clay apex. Thus a three-dimensional figure is obtained whose height at any point, corresponding to a given individual soil sample correctly placed on the plane of the inscribed base, provides the moisture equivalent of the soil sample concerned. This is simply an extension of the idea, experimentally established by the present results, that the moisture equivalent of a soil mixture is represented by some value intermediate between the moisture equivalents of the two soil components, and of a magnitude dependent upon the proportions of those components present in the mixture. In figure 2 the moisture equivalent for a mixture of sand and silt containing x per cent silt is given by the vertical distance y. Similarly the height y' gives the moisture equivalent of a mixture of sands and clay represented by the point E. Soils of intermediate composition between D and E, such as F, will have moisture equivalents between y and y'. Soil F, for example, would have the moisture equivalent y''. By location along A'B', B'C', and A'C' of the possible moisture equivalent values, points of equal height in the plane A'B'C' may be connected by straight lines. A moisture equivalent scale is thus obtained upon the sloping area which may be projected conveniently upon the horizontal base, as in figure 3, whereby a diagram is obtained providing for the rapid, direct, graphic calculation of the moisture equivalent of a soil sample having a given textural composition. Conversely, given the moisture equivalent and the amount present of any one of sands, silt, or clay, the mechanical analysis and hence the class name may be determined graphically.

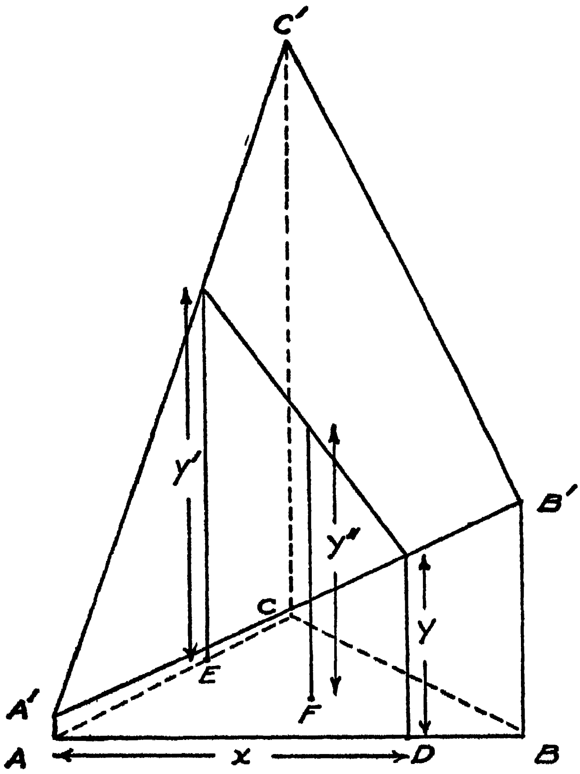

FIG. 2. DIAGRAM ILLUSTRATING RELATION BETWEEN MOISTURE EQUIVALENT AND MECHANICAL COMPOSITION

The efficiency of the method outlined as a means of obtaining the textural class name of a soil sample was tested for 169 surface and subsoil samples from three soil survey areas in California. In order to obtain in addition a more representative set of soils for consideration reference was made to the paper by Middleton (11) which includes mechanical analyses and moisture equivalents for 34 soils from various parts of the country. Textural class names were then assigned by means of a larger scale, modified form of figure 3, a moisture equivalent-sand diagram, in which percentage of sands was marked off along the horizontal axis and percentage moisture equivalent along the vertical axis. The boundary lines of the areas representing the various textural classes were then drawn in through those coördinate points representing transitions from one textural class to another. The values for sand content and moisture equivalent were obtained and the class name was decided from the area in which the points of intersection fell. Any points falling outside the diagram were assigned to the class whose area they most nearly approached. Of the Californian samples examined, 100 were analyzed by the centrifugal method of separation and 69 by the pipette method following acetic acid, hydrogen peroxide, hydrochloric acid, and sodium oxalate pretreatment (12). The results are given in table 3. "Complete agreement" signifies that the same class name would be given to the soil sample whether the name were determined by means of the triangular mechanical analysis class diagram of Davis and Bennett, or by the moisture equivalent-sand diagram. When the two methods

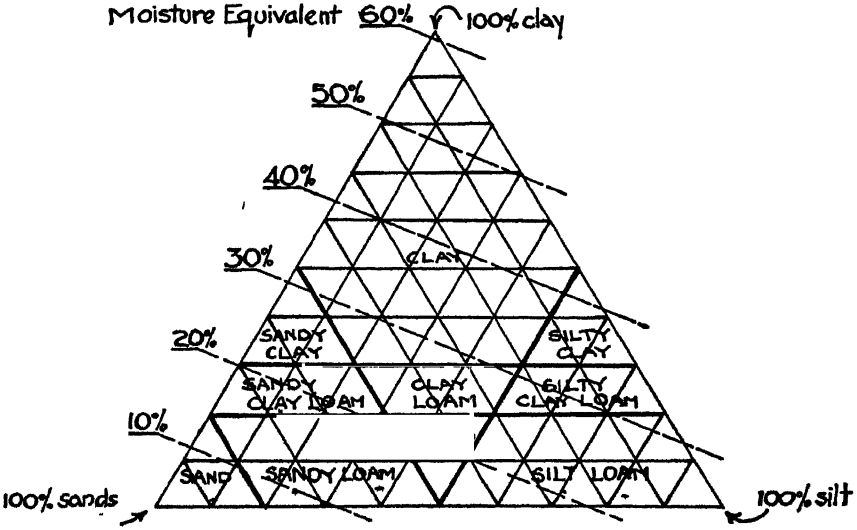

FIG. 3. SOIL CLASS-MOISTURE EQUIVALENT DIAGRAM BASED ON DAVIS' AND BENNETT'S TRIANGLE AND SMITH'S MOISTURE EQUIVALENT VALUES FOR SOIL SEPARATES

of obtaining the texture name resulted in the names of adjacent class areas being assigned to a single sample the agreement was considered as "fair." In some cases the soils showing "fair" agreement were actually very near to complete agreement, and separated from each other by only a short distance, yet falling in two different classes. When a greater discrepancy than the foregoing was obtained the agreement was termed "poor."

A moisture equivalent-sand diagram method of textural classification may be expected to attribute more weight to the field behavior of the soil than does the mechanical analysis, since two properties have been used which are associated closely with field methods of classification and field behavior and which may be expected considerably to influence the judgment of the field observer. This probably explains the higher degree of agreement between the mechanical analysis and moisture equivalent methods of class naming when the centrifugal method of mechanical analysis was used than was obtained by means of the pipette method of analysis. The analytical method giving the higher degree of dispersion probably overemphasized the fineness of the soil from the field point of view. The class designations in the case of the diagram of Davis and Bennett are based on centrifugal mechanical analysis of the soils already named texturally by experienced men who collected them (6). This diagram can hardly be expected to continue to serve as the basis of textural classification if a method of analysis is used which no longer has the field significance of that upon which the diagram itself is based.

TABLE 3

Degree of agreement obtained between class names determined by triangular diagram (Davis and Bennett) and moisture equivalent-sand diagram (based on Smith's values)

AGREEMENT	AMMONIA PRETREATMENT—CENTRIFUGAL SEPARATION		PEROXIDE-ACID-SODIUM OXALATE PRETREATMENT—PIPETTE SAMPLING AND SIEVING
	Californian soils	Middleton's soils	
	per cent	*per cent*	*per cent*
Complete	56	65	29
Fair	38	35	39
Poor	6	0	32

SUMMARY

The results of moisture equivalent determinations of mixtures of soil samples of known moisture equivalent values and in known proportions are presented in support of the suggestion that a single diagram based upon Davis' and Bennett's soil class triangle, Smith's directly determined moisture equivalent values of soil separates, and the total sand content, may be used to give useful approximations to the textural class names. The texture of the sample would thus be fixed by means of moisture equivalent and total sand determinations only, both being operations which can be performed rapidly. In addition, the highly useful moisture equivalent value would be available for various other purposes. Since the diagram of Davis and Bennett is in turn based upon the mechanical composition of the soil as analyzed by a mild form of pretreatment and centrifugal separation (the Bureau of Soils old method of analysis), it is evident that this method of analysis alone can be used for an accurate designation of the class names of normal soils where this particular triangular diagram is to be used.

The purpose of the mechanical analysis of samples collected during the course of a soil survey is primarily to guide the soil surveyors in their application of textural names and secondarily to indicate the probable field behavior of the soil. It is not to be expected, therefore, that a method of analysis, the object of which is complete dispersion of the soil, will be well fitted to the needs of a soil survey, although such a method has much to recommend it for soil physics researches and detailed profile examinations.[1]

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INVESTIGATIONS CONCERNING SEPARATION OF SIMILARLY CHARGED IONS FROM SOILS BY ELECTRODIALYSIS[1]

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In electrodialysis experiments the different cations which are liberated from the solutions or suspensions contained in the middle compartment of a three-chambered apparatus will be accumulated in the cathode chamber as hydroxides. If the material which is dialyzed contains a compound of cations that can be liberated, for example K, Na, Ca, Mg, Fe, and Al, the cathode water after some time will consist of a mixture of the respective hydroxides, of which the hydroxides of magnesium, iron, and aluminum will precipitate more or less completely, because they are relatively insoluble in alkaline solution. The formation of these hydroxides can, if more than traces are present, be distinguished since the cathode water changes from colorless to light yellow.

The anions liberated from an electrodialyzed medium migrate to the anode where a formation of acids in the anode water takes place. If we deal with soil suspensions which contain humus material the dialyzate in the anode chamber will consist of a mixture of mineral acids and electrodialyzable "humic acids." The presence of "humic acids" will make the color of the anode water more or less yellow or brown, depending on the amount which enters the anode chamber. In the electrodialysis of muck or peat soil the anode water may be dark brown.

The analytical procedure for separation of the different ions in the dialyzates obtained by electrodialysis of soils is relatively simple compared with the procedure of analysis of extracts from leaching experiments. This is because of the greater degree of purity of the dialyzates as compared to the extracts obtained by treating the soil samples with acids or solutions of neutral salts. If it were possible, however, to separate the different ions in the apparatus itself, before the dialyzates were drained from the respective chambers, it would be of great theoretical interest and also have considerable practical value, since it would simplify the analytical procedure. Such a separation of equally charged ions would find application in different branches of science, and not alone in the investigation of soils.

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The work reported in this paper was performed by the author while located in the soils department of Ohio State University, under a fellowship supported by the Rockefeller Foundation. The author gratefully acknowledges the helpful suggestions and criticisms given by Dr. R. Bradfield during the progress of the investigations.

The possibility of an electrodialytical separation of ions bearing similar electrical charge has, according to the author's knowledge, never been discussed in the literature. The idea that at least a partial separation of the ions liberated from soils could be obtained, arose in connection with a series of electrodialysis previously performed by the author (3). The fact that some of the liberated ions form, and some ions do not form, precipitates in the electrode chambers, was the fundamental principle upon which the idea of separation was based.

APPARATUS AND METHODS OF INVESTIGATION

In order to test the possibility of a separation of different cations and different anions the author has constructed a five-compartment electrodialysis apparatus, which is shown in plate 1.[3] This apparatus was developed from the author's previously described three-compartment cell (2) by inserting two new chambers between the middle chamber and the end chambers, one chamber at each side. These intermediate chambers have a capacity of 60 to 65 cc. or about half the volume of the end chambers. It is possible that the volume of the intermediate chambers may later be changed so that all chambers, except the middle chamber, will be of equal capacity. However, the intermediate chambers should be made short so that the distance between the electrodes, which are placed in the end chambers, is not greatly increased. Like the other glass parts of the apparatus, the new chambers are made of Pyrex glass.

A five-compartment apparatus, or a multiple cell with more than three compartments, can easily be constructed from the three-compartment cell. The requirements are only extra chambers and an extra set of sufficiently long rods, which are fitted to the frame of the three-compartment apparatus. As it is convenient to have supports beneath the connections of the chambers when the different parts are joined together, small supports of wood have been made. These are so formed that they fit to the flanges of the compartments which are placed together. The many connections in a multiple-compartment cell require a rather high pressure from the adjusting screw in order to get all chambers tight. Therefore all gaskets between the flanges and the cushions at the ends of the apparatus must consist of soft rubber.

A separation of different cations and different anions in the multiple apparatus has been investigated according to the following principles:

(a) By using different kinds of membranes between the different chambers.

(b) By precipitation of some of the liberated cations in the intermediate chamber (C. I) whereas other cations are allowed to migrate to the end chamber (C. II).

(a) With regard to the action and the permeability of membranes, Weiser (7) has reviewed and discussed the different theories which deal with this problem. Stated briefly, he has pointed out that separation of different substances by membranes appears to depend partly on:

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[3] This apparatus can be supplied by the Central Scientific Company of Chicago.

A. The size of the pores in the membranes in relation to the size of the ions or molecules which are to be separated.

B. Solubility of the substances in the membranes

C. Adsorption in the membranes, the latter acting as adsorbents which take up more of some substances than they do of other substances. If the solvent is adsorbed to a greater extent than the solute we have the so-called negative adsorption

Then, the penetration of ions or molecules through membranes is more or less affected by the nature of the electrical charge on the membranes themselves, as briefly discussed in a previous publication (3).

Compared with ordinary dialysis the penetration of ions or molecules through the membranes is more or less forced because of the application of the electrical current. The case is therefore rather complicated and it is difficult to suggest before hand the types of membranes by which a separation of the similarly charged, but different ions can be obtained.

(b) The separation of different ions by precipitation of some of the ions in the intermediate chamber seems more simple when dealing with a mixture of various cations, because of the insolubility of certain hydroxides.

How far a separation of similarly charged ions can be obtained by arranging electrodes in all chambers of the multiple cell and by a regulation of the drop in potentials between the electrodes has not been investigated. By such an arrangement it would be possible to decrease the distance between the electrodes, and the process of electrodialysis would be facilitated. Then the interesting problem of separating different ions according to the size of the electrical charge on the ions could be studied.

In the following pages results are presented from a series of experiments in which an electrodialytical separation of similarly charged ions has been attempted. The experiments performed are of a preliminary character, and it was the author's intention not to present the multiple electrodialysis apparatus till more extensive investigations were made. Several circumstances, however, have made it impossible to continue at the present time and therefore it seems desirable to publish the results which already have been obtained.

EXPERIMENTAL

The experiments reported in the following paragraphs were performed with 125 volts. The distance between the electrodes, which were placed in the end chambers, varied according to the number of compartments used in the different experiments. In the three-compartment cell the electrode distance was 9 cm.; for each new compartment inserted the distance was increased by 3 cm. so that we have 12 cm., 15 cm., and 18 cm. electrode distance when four-, five-, or six-compartment cells were used, respectively. Four compartments were used when a separation of only one part of the ions liberated from the medium electrodialyzed was desired, and five compartments when a separation of both the cations and anions was the object to be attained. In a few cases an apparatus consisting of six compartments was tried.

As will be understood, the potential differences were small when four or more compartments were used, and the removal of ions was accordingly slow. In order to speed up the movement of ions the voltage should be increased, which also would increase the amperage, and when soils are to be electrodialyzed this is to be recommended.

The quantitative analyses were carried out according to standard methods: Silica was determined as SiO_2, iron and aluminum as $Fe_2O_3 + Al_2O_3$, iron alone by reduction with stannous chloride and subsequent titration with $KMnO_4$, magnesium as $Mg_2P_2O_7$, and sodium + potassium as the sum of the chlorides. The nitrate nitrogen was determined with the phenoldisulfonic acid method, phosphoric acid was determined volumetrically as ammonium-phosphomolybdate, chlorine was titrated with $AgNO_3$ solution, and sulfate was determined (approximately) with the turbidity test of the barium salt.

The qualitative tests of Fe^{+++} were made by the thiocyanate method, Fe^{++} was detected by potassium ferricyanate, aluminum with alizarin-S (sodium salt of alizarin monosulfonic acid), calcium as oxalate, magnesium as pyrophosphate, nitrate with diphenylamine, phosphate with stannous chloride in solution of ammonium molybdate, chlorine as silver chloride, and sulfate as barium sulfate. In a few samples manganese was expected to be present and tests for Mn with potassium periodate were made. [Descriptions and references to the different methods can be found in Yoe (8).]

Experiments with pure salt solutions

In order to select the types of membranes which might be suitable for use in the separation of certain ions a series of experiments were performed with pure salt solutions. Results from these experiments are given in tables 1, 2, and 3.

The experiments reported in tables 1 and 2 were made with the three-compartment cell. In table 1 are given results from experiments with solutions of ferric sulfate and calcium chloride. The purpose of these experiments was to find a membrane by which iron could be separated from calcium. The results can be summarized briefly as follows:

Fishskin, parchment, and cellophane membranes are all permeable to Ca and Fe^{+++}; rubber membrane, however, is not permeable to Fe^{+++} under ordinary experimental conditions. The rubber membrane allows penetration of Ca but the process is very slow.

When rubber was used as the cathode membrane a precipitate of iron was found on the middle chamber side of the membrane. In the other experiments iron precipitate was found in the cathode membranes themselves, but the membranes were still permeable to the ferric ion, since $Fe(OH)_3$ was detected in the cathode water. The formation of precipitate on and in the membranes must be due to hydrolysis around the membranes. This explanation is based on the statement made by Bradfield (1) that when a solution is electrodialyzed

TABLE 1

Experiments with different kinds of membranes and solutions of ferric sulfate and calcium chloride in the three-compartment apparatus

EXPERIMENT NUMBER	TIME ELECTRODIALYZED	TEMPERATURE	IONS DETECTED IN THE CATHODE CHAMBER (C.)	MEMBRANE	SOLUTIONS ELECTRODIALYZED	MEMBRANES	IONS DETECTED IN THE ANODE CHAMBER (A.)	NOTES
	hours	°C.						
1	2	30	Fe^{+++}	Fishskin	0.2 *N* $Fe_2(SO_4)_3$ solution	Fishskin	SO_4^{--} Fe^{+++}	In C. heavy precipitate
2	2	30	Fe^{+++}	Parchment	0.1 *N* $Fe_2(SO_4)_3$ solution	Parchment	SO_4^{--} Fe^{+++}	In C. heavy precipitate
3	2	60	Ca^{++}	Rubber	0.1 *N* $Fe_2(SO_4)_3$ and 0.05 *N* $CaCl_2$ solution	Parchment	Cl^- SO_4^{--} Fe^{+++}	Precipitate of Fe on the cathode membrane
4	2	100	Ca^{++} Fe^{+++} Fe^{++} (trace)	Parchment	0.1 *N* $Fe_2(SO_4)_3$ and 0.05 *N* $CaCl_2$ solution	Rubber	Cl^- SO_4^{--} Fe^{+++} (trace)	Precipitate of Fe on the cathode membrane
5	50	30	Ca^{++}	Rubber	0.1 *N* $Fe_2(SO_4)_3$ and 0.05 *N* $CaCl_2$ solution	Rubber	Cl^- SO_4^{--}	Very slow ion migration
6	1	40	Fe^{+++}	Cellophane	0.1 *N* $Fe_2(SO_4)_3$ solution	Cellophane	SO_4^{--} Fe^{+++}	Precipitate of Fe on the cathode membrane

TABLE 2

Experiments with different kinds of membranes and solutions of different chlorides in the three-compartment apparatus

EXPERIMENT NUMBER	TIME ELECTRODIALYZED	TEMPERATURE	IONS DETECTED IN THE CATHODE CHAMBER (C)	MEMBRANES	SOLUTIONS ELECTRODIALYZED	MEMBRANES	IONS DETECTED IN THE ANODE CHAMBER (A.)	NOTES
	hours	°*C.*						
1	6	35	Fe^{+++}	Parchment	0.1 *N* $FeCl_3$ solution	Parchment	Cl^- Fe^{+++}	Precipitate of Fe on the cathode membrane
2	3	35	Fe^{+++}	Cellophane	0.1 *N* $FeCl_3$ solution	Cellophane	Cl^- Fe^{+++}	Heavy precipitate of Fe on the cathode membrane
3	6	35	Al^{+++}	Parchment	0.1 *N* $AlCl_3$ solution	Parchment	Cl^- Al^{+++}	Precipitate of Al on the cathode membrane
4	3	50	Al^{+++}	Cellophane	0.1 *N* $AlCl_3$ solution	Cellophane	Cl^- Al^{+++}	Heavy precipitate of Al on the cathode membrane
5	1	100	Mg^{++} (heavy precipitate)	Parchment	0.1 *N* $MgCl_2$ solution	Parchment	Cl^- Mg (trace)	Precipitate of Mg on the cathode membrane
6	1	35	Mg^{++} (heavy precipitate)	Cellophane	0.01 *N* $MgCl_2$ solution	Cellophane	Cl^- Mg (trace)	Precipitate of Mg on the cathode membrane

TABLE 3

Experiments with different kinds of membranes in the five-compartment apparatus

EXPERIMENT NUMBER	TIME ELECTRODIALYZED	TEMPERATURE	IONS DETECTED IN C. II	MEMBRANES	IONS DETECTED IN C. I	MEMBRANES	SOLUTIONS ELECTRODIALYZED (MIDDLE CHAMBER)	MEMBRANES	IONS DETECTED IN A. I	MEMBRANES	IONS DETECTED IN A. II	NOTES
	hours	°C.										
1	2	35	Fe^{+++}	Fish-skin	Fe^{+++}	Fish-skin	0.1 *N* $Fe_2(SO_4)_3$ solution	Fish-skin	SO_4^{--} Fe^{+++}	Fish-skin	SO_4^{--} Fe^{+++}	
2	2	100	Fe^{+++} Fe^{++} Ca^{++}	Cellophane	Fe^{+++}	Cellophane	0.05 *N* $Fe_2(SO_4)_3$ and 0.02 *N* $CaCl_2$ solution	Cellophane	SO_4^{--} Fe^{+++}	Cellophane	Cl^- SO_4^{--} Fe^{+++}	No cooling water applied
3	2	50	Na^+	Cellophane	Fe^{+++} (NaOH added)	Cellophane	0.05 *N* $Fe_2(SO_4)_3$ solution	Cellophane	SO_4^{--} Fe^{+++}	Cellophane	SO_4^{--} Fe^{+++}	In all 10 cc. of 0.2 *N* NaOH added in C. I to keep the reaction alkaline during the experiment. Portions of 2 cc. were used
4	20	35	Ca^{++} NH_4^+	Rubber	Ca^{++}	Cellophane	0.1 *N* $CaCl_2$ and 0.1 *N* $(NH_4)_2HPO_4$ solution	Cellophane	PO_4^{---}	Rubber	Cl^- PO_4^{---}	Precipitate of PO_4 complex on the anode rubber membrane

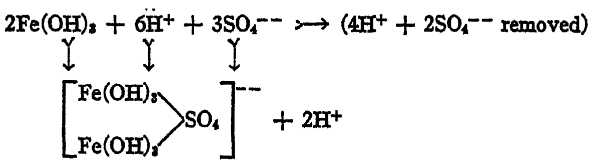
hydrolysis takes place, forming an alkaline reaction around the cathode membrane and an acid reaction around the anode membrane.

To the author's surprise iron was found in significant amounts in the anode fractions from all experiments where rubber was not used as anode membrane. That Fe^{+++} as an electropositive ion migrates to the anode seems very improbable, and the explanation why iron is found in the anode fractions must be that a complex ion with electronegative charge is formed. It is known that the sesquioxides of iron and aluminum are electrical ampholytes. The corresponding hydroxides are supposed to be electropositively charged, but, as Mattson (4) has pointed out, these hydroxides might under certain conditions contain more or less acid anions. If ferric hydroxide is taken as an example the $Fe(OH)_3$ molecule combines in water with the OH ion, the complex being negatively charged, and migrates toward the anode. The H ion, however, remains free and migrates to the cathode.

Now the question becomes pertinent if a formation of $Fe(OH)_3$ is probable or possible in the solutions used in the electrodialysis experiments. First it should be stated that the movement of iron toward the anode did not take place immediately when the electrical circuit was established but only after prolonged electrodialysis when a considerable amount of the acid anions was removed from the solution. If a solution of $Fe_2(SO_4)_3$ is electrodialyzed the following reactions might take place:

$$Fe_2(SO_4)_3 \rightleftharpoons 2Fe^{+++} + 3SO_4^{--}$$

$$6HOH \rightleftharpoons 6OH^- + 6H^+$$



$$\begin{array}{l} 2Fe(OH)_3 + 6H^+ + 3SO_4^{--} \rightarrowtail (4H^+ + 2SO_4^{--} \text{ removed}) \\ \quad\ \downarrow \qquad\quad \downarrow \qquad\quad \downarrow \\ \left[\begin{matrix} Fe(OH)_3 \\ Fe(OH)_3 \end{matrix} \Big\rangle SO_4 \right]^{--} + 2H^+ \end{array}$$

The foregoing equation indicates that some of the H and SO_4 ions will be removed by electrodialysis and at a certain point of acidity in the solution the $Fe(OH)_3$ molecules formed might absorb SO_4 ions remaining in the solution. The pH value of the solution when the migration of iron toward the anode starts has not been exactly fixed, but tests with litmus paper showed that the reaction was slightly acid.

The suggested ferric-hydroxide-sulfate complex, which could be illustrated by the formula

must be of molecular dimensions, since it penetrates through certain types of membranes. Rubber membrane, however, seemed impermeable for this complex, and only under extreme conditions, as when the temperature in the apparatus arose to the boiling point, which might influence the behavior of the membrane considerably, a trace of Fe^{+++} was detected in the anode water.

Experiment with a solution of ferrous sulfate (not reported in the tables) showed that iron migrates toward the anode also in the ferrous form.

A migration of iron to the anode is observed also in case a $Fe\ Cl_3$ solution is electrodialyzed, but it takes place to a less extent than when a $Fe_2(SO_4)_3$ solution is used. The reason must be that the ferric hydroxide complex combines with the divalent SO_4 ion to a greater extent than it does with the monovalent Cl ion [cf. Mattson (4)].

Solutions containing aluminum and magnesium chlorides have also been investigated in order to determine whether the aluminum and magnesium show a behavior similar to that of iron in the electrodialytical system. It was found that both the aluminum and the magnesium migrated to the anode but in the case of $MgCl_2$ solution only a trace of Mg-ions was detected in the anode fractions, whereas in the cathode fractions a heavy precipitate of $Mg(OH)_2$ was formed. This was the case regardless of whether the temperature was 35° or 100°C.

Also in the experiments with aluminum and magnesium solutions a precipitate was found on the cathode membrane. The experiments, which are reported in table 2, show that parchment and cellophane are permeable to Al^{+++} and Mg^{++}.

According to information given to the author by R. Bradfield a movement of aluminum and magnesium to the anode has also been observed in his electrodialysis experiments. The data, however, have not yet been published.

In table 3 are reported results of experiments with different kinds of membranes in the five-compartment apparatus. Experiment 1 shows that iron is found in all the side chambers when fishskin is used as membranes. The movement of iron, however, is rather slow, since a considerable amount was found in the intermediate chambers.

In experiment 2 the temperature was allowed to rise to the boiling point. Under these experimental conditions also, iron moved to the end cathode chamber together with calcium when cellophane was used as membrane between C. I and C. II. A certain amount of ferric ions was reduced to ferrous ions. A reduction of the ferric to the ferrous form was also observed in experiment 4, table 1, where the temperature reached the boiling point.

Experiment 3 differs from the others in this respect in that small portions of NaOH solution were added to the dialyzate in C. I during the time of experimentation. The purpose of such a procedure was to study whether or not it was possible to precipitate all the iron as hydroxide in C. I and prevent its movement to C. II. The reaction in C. I was observed by introducing litmus paper in this compartment. As is seen from table 3 no iron was detected in C. II in this experiment. A relatively strong alkaline reaction in the intermediate chamber should be sufficient to prevent the movement of iron to C. II, regardless of whether or not the membrane used between C. I and C. II is permeable to iron. From experiment 2 it is seen that the transportation of calcium through C. I has not been sufficient protection to stop the iron from penetrating through the cellophane membrane to C. II, but the high temperature in that experiment might have forced the migration to a certain extent.

Experiment 4 dealt with a calcium chloride and diammonium phosphate solution. As seen from the table the rubber membrane is rather impermeable to PO_4 ion since PO_4 is detected in A. I after 20 hours of dialysis. The presence of a small amount of Ca in C. I shows that rubber also is somewhat impermeable to that ion. By repeating this experiment and electrodialysis during a period of 160 hours all PO_4 and Ca ions were removed from the intermediate chambers.

An experiment was also performed with a solution of $0.1N$ $CaCl_2$ and $0.1N$ $FeCl_3$ solution, using a six-compartment apparatus of which three compartments were placed on the cathode side and two on the anode side. The experiment was continued for 3 hours at a temperature of 30°. Parchment was used as membranes between all chambers. Qualitative tests showed that practically all the Ca was removed to the end chamber (C. III) only a trace was detected in C. I, whereas a heavy precipitate of Fe was found in this chamber. In C. II only a trace of Fe was present, but Fe was detected in significant amounts in both anode chambers.

Experiments with different types of soils

Experiment with Bedford red clay from Central College, Franklin County, Ohio, in the five-compartment apparatus. A 50-gm. soil sample which had passed through a 100-mesh sieve was used. The temperature was held at 30°C. As membrane cellophane was used between the middle compartment and the two intermediate chambers. Between the last mentioned chambers and the end chambers rubber membranes were used.

As the ammeter reading indicated that the liberation and removal of ions from the soil suspension was very slow, presumably mostly because of high resistance in the rubber membranes, dilute HCl was added in the middle chamber in order to improve conductivity. The reaction of the soil suspension, which was slightly alkaline was then changed to about pH 3. In all, 40 cc. $0.1N$ HCl were required to reach the pH value mentioned. After 48 hours of dialysis the dialyzates were drained from all chambers.

In the dialyzate from C. I a slightly white-yellow precipitate was observed, whereas the dialyzate from the end chamber (C. II) was perfectly clear and colorless. Quantitative analysis of the two fractions showed that the dialyzate from C I contained 4.35 m.e. of Mg and 0.23 m.e. of Mn per 100 gm. of soil, besides a small amount of Si but no Fe or Al.[4] Neither Ca, K, or Na was found in the dialyzate from C. I. The dialyzate from C. II contained 10.36

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[4] The presence of Si in the cathode water was first observed by Odén (5) and later has been found by several investigators.

m e. of Ca and 1.76 m.e. of K + Na per 100 gm. of soil. In this fraction no traces of Mg, Mn, Fe, Al, or Si were found.

These results seem to indicate that Mg and Mn can be separated from Ca, K, and Na under certain conditions but they do not tell whether the separation is a result of impermeability of the rubber membrane for the ions mentioned, or whether it is due to precipitation of Mg and Mn as insoluble hydroxides in the slightly alkaline solution which is formed in the intermediate chamber (C. I) as a result of the passage of Ca, K, and Na. On the contrary, the separation of Si from Ca, K, and Na might be the result of a membrane effect, since conditions in C. I should not promote a precipitation of Si in this chamber.

The anode fractions, both of which were perfectly clear and colorless, were not analyzed. It was proved, however, that the Cl ions from the added HCl moved to the end chamber (A. II) and, at least to a certain extent, were liberated as chlorine gas.

Experiment with a sample of Paulding clay, subsoil, from Paulding County Experimental Farm, Ohio. The experimental conditions varied from those described in the previous experiment in these respects: The rubber membranes were changed and instead parchment was placed between the intermediate chambers and the end chamber. No HCl was added to the soil suspension. Every 24 hours the dialyzates were drained and the chambers refilled with distilled water. The experiment was continued for 168 hours.

Qualitative determinations made in the dialyzates confirmed the fact that the cations which form strong bases move to the electrode chamber (C. II), and that the cations which form insoluble hydroxides will at least to a large extent precipitate in the intermediate chamber regardless of whether or not the membrane which is placed between these chambers is permeable to these ions. However, if the intermediate chamber is drained while the transportation of ions is proceeding, small amounts of certain ions which otherwise would be found in the end chamber might be found in the intermediate chamber. This was proved in this experiment, as the first fraction of the dialyzate from C. I contained a small quantity of Ca.

The anode fractions were tested for phosphoric acid colorimetrically. It is of interest to note that none of the fractions from A.I contained PO_4 ion. The first fraction from A. II was found to be high in phosphoric acid content; on the contrary the PO_4 in the second fraction was very low. In the third and fourth fractions the PO_4 content was medium and then it gradually decreased from fraction to fraction and by the end of 168 hours of dialysis only traces were found.

The pH value of the original soil suspension was 6.60. At the end of the second interval, which introduced the second maximum of PO_4 liberation, the pH value of the suspension was 5.35, and by the end of the fourth interval it was 4.95. In the liberation of cations corresponding increases in the amounts removed from the soil during the third and fourth intervals were found.

Experiment with a sample of Saw grass muck soil from the Everglades Experiment Station, Belle Glade, Florida.[5] The experiment was performed under almost the same conditions as those described in experiment 1. The size of the sample used for electrodialysis, however, was 25 gm., and dilute HCl was not added during the first interval of 48 hours (a). The membrane arrangement is shown in table 4. The pH value of the soil suspension was 5.65 at the start and 5.25 at the end of the first interval. After the dialyzates had been changed the experiment was continued for a new period of 48 hours during which a total of 30 cc. of $0.1N$ HCl was added in several portions (b). The pH of the suspension was then changed to about 5. The main purpose of this experiment was to determine whether or not a separation of the electrodialyzable humus acids from the mineral acids could be made.

The anode fractions from A. II were perfectly clear and colorless, whereas the a-fraction from A. I was clear but yellow and the b-fraction from the same chamber was clear but dark brown. The rubber membrane placed between A. I and A. II had evidently caused the "humic acids" liberated from the suspension in the middle chamber to be retained in A. I.

The analytical data from the experiment are given in table 4. It is of interest to note that all strong mineral acids are found in A. II. The phosphoric acid, however, is found in both A. I and A. II, but in greatest amounts in A. I. Rubber membrane is, in other words, permeable to PO_4 ions to a certain extent, as also was found in the experiment with diammonium phosphate solution (table 3, experiment 4).

In all anode fractions traces of silica were detected. According to this, rubber is not entirely impermeable to silica, at least in an acid medium. No traces of Fe + Al were found in the anode fractions.

Comparisons between the sums of mineral acids, in terms of milligram equivalents, found by chemical analysis and the amounts of acids found by titration show that there is a significant difference in the a-fraction from A. I and a rather great difference in the b-fraction from the same chamber. These differences must presumably be "humic acids" which are held back in A. I by the rubber membrane together with phosphoric acid. Similar comparisons made for the anode fractions from A. II show that the amounts found by analysis and by titration agree very well with each other.

It should be stated that the titrations of the A. I-samples were made on aliquots sufficiently diluted so that the color due to the "humic acids" did not interfere seriously with the color of the indicator used (methyl red).

In order to compare the amounts of organic material in soluble form in the different anode fractions aliquots were treated with dilute sulfuric acid and then titrated with $KMnO_4$ solution. The amounts of permanganate solution used for oxidation of the organic material should indicate whether or not the separation of the electrodialyzable "humic acids" from the mineral acids, except

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[5] The author gratefully acknowledges the coöperation of Mr. J. A. Naftell who furnished this sample.

TABLE 4

Experiment with 25 gm. of a muck soil electrodialyzed in the five-compartment apparatus

FRACTIONS	M.E. IONS IN CATHODE CHAMBERS				MIDDLE CHAMBER	M.E. IONS IN ANODE CHAMBERS			
	C. II	Membrane	C. I	Membrane		Membrane	A. I	Membrane	A. II
a	No Fe + Al Ca = 2.707 Mg = 0.099 K + Na = 0.301 Sum = 3.107 Titratable bases = 3.277 Si = 0.799	Parchment	No Fe + Al Ca = 0.307 Mg = 0.119 No K + Na Sum = 0.426 Titratable bases = 0.229 Si = 0.899	Parchment	25 gm. of muck soil suspended in 450 cc. volume	Cellophane	Si (trace) PO_4 = 0.021 No NO_3 No SO_4 No Cl Sum = 0.021 Titratable acids = 0.252	Rubber	Si (trace) PO_4 (trace) NO_3 = 0.388 SO_4 = 0.300 Cl = 0.145 Sum = 0.833 Titratable acids = 0.890
b	No Fe + Al Ca = 6.617 Mg = 0.119 K + Na = 0.421 Sum = 7.157 Titratable bases = 7.012 Si = 0.799		No Fe + Al Ca = 0.639 Mg = 0.118 K + Na (trace) Sum = 0.757 Titratable bases = 0.508 Si = 0.166		In all 30 cc. of N/10 HCl added to the soil suspension		Si (trace) PO_4 = 0.106 No NO_3 NO SO_4 No Cl Sum = 0.106 Titratable acids = 0.804		Si (trace) PO_4 (small amount) NO_3 = 0.228 SO_4 = 0.080 Cl = 1.074 Sum = 1.382 Titratable acids = 1.513

phosphoric acids, is complete. It should be pointed out that no ferrous salts or other reducing substances which could interfere with the results were present in the anode fractions. In the following collocation the results are given in cc. $0.05N$ $KMnO_4$ solution per fraction:

The a-fractions: A. I used 7.2 cc. and A. II used 0.0 cc. $KMnO_4$
The b-fractions: A. I used 46.6 cc. and A. II used 0.0 cc. $KMnO_4$

The data show that the rubber membrane, under the experimental conditions stated in the foregoing, has been entirely impermeable to "humic acids." The effect of the HCl treatment of the soil suspension during the b-interval on the liberation of "humic acids" from the soil studied is indicated in the great difference between the amounts used for oxidation of these acids in the two fractions.

The results concerning the cations liberated from the same soil are also given in table 4. No Fe + Al are found in any of the fractions. The electrodialysis of the sample was continued only until about pH 5 was reached in the suspension. It was found in earlier experiments that the liberation of Fe and Al takes place only when the reaction of the suspension has changed to below pH 5 (6).

As seen from the table calcium is found in the largest amounts in the C. II fractions but small amounts are also found in the fractions from C. I. The presence of Ca in C. I can easily be understood, as the liberation of Ca from the suspension still was proceeding when the cathode chambers were drained. In order to obtain complete movement of Ca to the end chamber, the middle compartment should be drained and refilled with distilled water some time before the end chambers are drained.

Magnesium was found in about equal quantities in all fractions, whereas potassium and sodium migrated readily to C. II. The trace of K + Na which was detected in C. I in the b-fraction was presumably there for the same reason as mentioned for Ca.

Silica was found to be present in all the cathode fractions. These results show that the parchment membrane used between C. I and C. II has not prevented the penetration of the silica complexes such as found in the case where rubber membrane was used on the cathode side (experiment with Bedford red clay).

Comparisons of the sums of cations found by analysis and the amounts found by titration gave relatively good agreement. These data also can be found in table 4.

The experiment with the Saw grass muck soil was repeated, the only difference in the experimental conditions being that a cellophane membrane substituted the rubber membrane between A. I and A. II. In this case the dialyzate in A. II becomes brownish in color presumably because of "humic acids," which, in this experiment, moved to the end chamber.

The result obtained in the experiment with the muck soil is of great interest. If it is not possible to provide a membrane more permeable to phosphoric acid but not permeable to "humic acids," it might be possible to produce pure "humic acids" by continued electrodialysis of the dialyzate from A. I, either in the same experiment (cf. the remarks to experiment 4, table 3), or in the three-compartment cell using rubber membrane on the anode side. Traces of silica apparently can be removed in the same way and the problem of producing the more or less hypothetical "humic acids" in pure form should be solved.

It also should be pointed out that the method of electrodialysis according to the principles developed in the foregoing might be very useful in the study of the different "humic acids" in the soil. By fractional electrodialysis the different acids which are liberated from the soil suspension at various pH values can be studied, and the effect of various treatments in the apparatus on the speed of acid liberation can easily be investigated.

Experiments with electrodialyzed colloidal clay later saturated with certain cations

In a series of experiments the removal and separation of ions from electrodialyzed colloidal Paulding clay later saturated with different cations were studied. The clay suspension, which was prepared and supplied by Dr. R. Bradfield, had a pH value of 3.45, contained 4.3 per cent of clay colloids, and the total base saturation capacity was known to be approximately 80 m.e. per 100 gm. of clay. The amount of suspension used in the experiments corresponded to 2 gm. of clay colloids, which were saturated with 6 parts of either Ca, Na, or K and with 2 parts of either Fe, Al, or Mg. The three first mentioned cations were added in the form of hydroxides, the three latter as solutions of the corresponding chlorides. In one case the Mg was added as oxide.

After being mixed the samples were shaken for 2 hours in a shaking machine and afterwards stored over night. Before pouring into the electrodialysis apparatus the samples were shaken thoroughly by hand, and the pH values determined. The suspension in the electrodialysis apparatus was thoroughly stirred and a constant temperature of 25°C. was maintained during the time of electrodialysis. The experiments were continued until the original pH value of the suspension was approximately reached.

The membrane arrangement in the experiments as well as the results are reported in tables 5, 6, 7, 8, and 9. The experiment reported in table 9 was performed with a four-compartment apparatus, as only one anode chamber was used, while the other experiments were made with the five-compartment cell, the two compartments at each side of the central chamber being used.

Table 5 gives the results with the Ca and Fe saturated clay. Practically all the Ca put into the soil suspension was found in C. II, and no trace was detected in C. I. The Fe liberated, however, was stopped in chamber C. I except possibly a trace in C. II. Only about half of the amount of Fe, however, which was used for saturation, was found in C. I. It should be noted that a considerable amount of Fe precipitate was found on and in the membrane between the middle chamber and C. I. Traces of silica were detected in all

TABLE 5

Experiment with electrodialyzed clay colloids later saturated with calcium and ferric ions and then electrodialyzed for 24 hours

CATHODE CHAMBERS				MIDDLE CHAMBER	ANODE CHAMBERS				pH OF THE SOIL SUSPENSION
C. II	Membrane	C. I	Membrane		Membrane	A. I	Membrane	A. II	
m.e.		*m.e.*				*m.e.*		*m.e.*	
Ca = 1.174 Si (trace)	Parchment	Fe (+Al) = 0.176 Si (trace)	Parchment	Clay suspension 1.200 m.e. Ca in $Ca(OH)_2$ 0.400 m.e. Fe in $FeCl_3$	Cellophane	Si (trace) Fe (+Al) (trace) Titratable acids = 0.017	Cellophane	Cl = 0.293 Si (trace) Fe (+Al) (trace) Titratable acids = 0.300	Initial 5.45 Final 3.45

TABLE 6

Experiment with electrodialyzed clay colloids later saturated with sodium and aluminum and then electrodialyzed for 24 hours

CATHODE CHAMBERS				MIDDLE CHAMBER	ANODE CHAMBERS				pH OF THE SOIL SUSPENSION
C. II	Membrane	C. I	Membrane		Membrane	A. I	Membrane	A. II	
m.e.		*m.e.*				*m.e.*		*m.e.*	
Na = 1.199 Si (trace)	Parchment	Al (+Fe) = 0.131 Si (trace)	Parchment	Clay suspension 1.200 m.e. Na in NaOH 0.400 m.e. Al in $AlCl_3$	Cellophane	Si = 0.299 Al (+Fe) (trace) Titratable acids = 0.030	Cellophane	Cl = 0.232 Si (trace) Al (+Fe) (trace) Titratable acids = 0.303	Initial 6.40 Final 3.60

TABLE 7

Experiment with electrodialyzed clay colloids, later saturated with potassium and magnesium and then electrodialyzed for 48 hours

CATHODE CHAMBERS				MIDDLE CHAMBER	ANODE CHAMBERS				pH OF THE SOIL SUSPENSION
C. II	Membrane	C. I	Membrane		Membrane	A. I	Membrane	A II	
m e. K = 1.199 Mg (trace) Fe + Al (trace) Si (trace)	Parchment	*m e.* Mg = 0.061 Fe + Al = 0.131 Si (trace)	Parchment	Clay suspension 1.200 m e. K in KOH 0.400 m.e. Mg in $MgCl_2$	Cellophane	*m e.* Si (trace) Fe + Al (trace) Mg (trace) Titratable acids = 0.030	Cellophane	*m e.* Cl = 0.244 Si (trace) Mg (trace) Titratable acids = 0.252	Initial 5.25 Final 3.50

TABLE 8

Experiment with electrodialyzed clay colloids, later saturated with potassium and magnesium and then electrodialyzed for 140 hours

CATHODE CHAMBERS				MIDDLE CHAMBER	ANODE CHAMBERS				pH OF THE SOIL SUSPENSION
C. II	Membrane	C. I	Membrane		Membrane	A. I	Membrane	A. II	
m e. K = 1.175 Mg (trace) Fe + Al (trace) Si (trace)	Parchment	*m.e.* Mg = 0.069 Fe + Al = 0.131 Si = 0.139	Parchment	Clay suspension 1.200 m e. K in KOH 0.400 m.e. Mg in MgO	Cellophane	*m e.* Si (trace) Fe + Al (trace) Mg (trace) Titratable acids = 0.010	Cellophane	*m e.* Si (trace) Fe + Al (trace) Mg (trace) Titratable acids = 0.035	Initial 5.20 Final 3.65

TABLE 9

Experiment with electrodialyzed clay colloids later saturated with potassium and magnesium and then electrodialyzed for 6 hours

CATHODE CHAMBERS				MIDDLE CHAMBER	MEMBRANE	ANODE CHAMBER	pH IN THE SOIL SUSPENSION
C. II	Membrane	C. I	Membrane				
m.e. K = 1.199 Mg (trace) Fe + Al (trace) Si (trace)	Cellophane	*m.e.* Mg (trace) Fe + Al (trace) Si (trace)	Parchment	Clay suspension 1.200 m.e. K in KOH 0.400 m.e. Mg in $MgCl_2$	Cellophane	*m.e.* Cl = 0.157 Si (trace) Fe + Al (trace) Mg (trace) Titratable acids = 0.151	Initial 5.22 Final 3.50

fractions and in the anode fractions were also detected significant traces of (Fe + Al). The amounts of titratable acids in the anode fractions are also given in the table. It will be seen that the amount of Cl in the A. II fraction accounts for the main part of the acids found in that chamber. The reason why a smaller amount of Cl is found in the anode water than the amount which was added in the $FeCl_3$ solution is that some of the Cl was liberated as gas at the anode. Practically no acids were found in A. I.

The experiment with the Na and Al saturated clay is reported in table 6. As the data easily can be studied in the table, and show principally results similar to those in the previously described experiment no further comment should be necessary.

The experiments recorded in tables 7 and 8 deal with K and Mg saturated clay. The experimental conditions were very much the same in the two cases, the only difference being that Mg was added as MgO in the latter instead of $MgCl_2$.

It is of interest to note that it required a much longer period of time to reach a low pH value in the case where MgO was used (table 8). In both experiments traces of Fe + Al and of Si were detected in all chambers. The slightly alkaline or neutral reaction in C. I has, in other words, not been sufficient to precipitate all Fe and Al as the respective hydroxides. As may be noted, traces of Mg were detected in C. II. The amounts found in C. I were very small in comparison to the amounts used for saturation. On the other hand, Mg seems to have a rather high replacing effect against Fe and Al, which were found in about the same amounts in these experiments as the amounts found when Fe or Al were used as saturation agents (table 5 and 6). It should here be stated that tests made showed that practically all the Fe + Al found in the Mg experiments consisted of Fe.

Table 9 gives the set-up and results of the experiment with the four-compartment cell. As membrane between C. I and C. II cellophane was used in order to determine whether the slightly lower permeability to cations which this type of membrane shows compared with parchment, would be sufficient to prevent the penetration of Fe, Al, and Mg under the existing experimental conditions. The experiment was continued for only 6 hours, which proved to be sufficient to reach the same pH value as in the experiment reported in table 7.

No trace of K was found in the intermediate chamber, but all had migrated to C. I. The membrane arrangement used, however, was not entirely satisfactory for a complete retention of Fe, Al, and Mg from the end chamber, since traces of these ions were detected in C. II. The amounts of Fe, Al, and Mg, however, which migrate to the second chamber from the central compartment seem to be very small even when the membrane which separates the two chambers is permeable to these ions. Especially is that found to be the fact when we deal with Fe and Al. This is not only the case in the last reported experiment but similar results may be noted when all experiments are taken into consideration.

SUMMARY

This paper deals with the possibility of an electrodialytical separation of ions bearing similar electrical charges.

A new five-compartment electrodialysis apparatus is presented.

Experiments performed indicate that a separation of certain ions can be made by using membranes of different permeability between the chambers which are placed on the same side of the central compartment.

Experimental results indicated that a separation of certain cations from other cations can apparently be made also by chemical means, as a precipitation can be arranged in an intermediate chamber, whereas other cations are allowed to migrate to the end chamber.

A method of producing pure "humic acids" is suggested.

It was found that Fe, Al, and Mg under certain conditions migrated to the anode, presumably in the form of complexes consisting of hydroxides which had absorbed acid anions.

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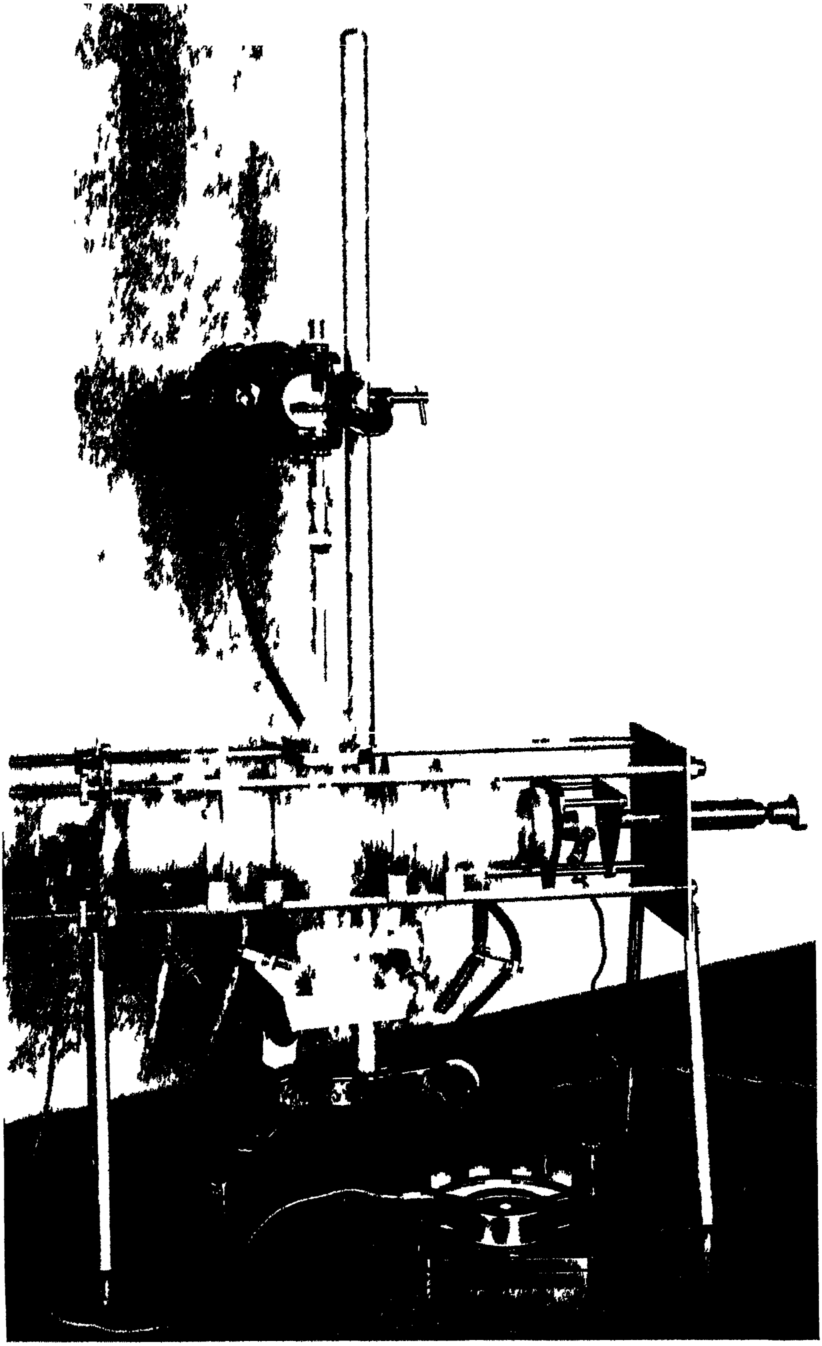
PLATE 1

FIVE-COMPARTMENT ELECTRODIALYSIS APPARATUS



INTERACTION BETWEEN AMMONIA AND SOILS AS A NEW METHOD OF DETERMINING THE STATE OF SATURATION AND pH VALUES OF SOILS

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In earlier publications (5, 6, 7), the writer has shown that the interaction between soil and ammonia represents the neutralization of acidoid by a base. It was also shown that a soil made completely unsaturated can combine with an amount of ammonia which may be considered equivalent to its base exchange capacity (7); also that the pH value of soils containing excess of $CaCO_3$, after dilute acid treatment and ammonia saturation, is approximately the same as that of the soil in the natural condition. In other words the titration curves of soil acidoids with $Ca(OH)_2$ and NH_4OH are almost identical.

Since pH values of natural soils represent single points on their titration curves, it is reasonable to suppose that the difference between the amount of ammonia taken up by a natural soil and the same soil completely unsaturated, will be a measure of the state of saturation of that soil and consequently will be correlated with its pH value. The object of this investigation was to explore the possibilities of this reaction for characterizing the state of saturation of soil.

EXPERIMENTAL

Ammonia reaction

The amount of ammonia that a natural soil can take up is determined as follows:

Ten to twenty grams of soil are taken in a dish and kept over normal ammonia in a vacuum desiccator for 2 days. It is then transferred to another desiccator and kept over 90 per cent H_2SO_4 for 2 days. The ammonia retained by the soil is then determined by distillation with lime in the usual way.

The maximum saturation capacity of the soil for ammonia is determined as follows:

Ten grams of soil is stirred with about 50 cc. of water. Enough $0.2N$ HCl to break up all the carbonates is added gradually and the mixture set aside with

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[1] This work was carried to completion by the help of a grant from the Imperial Council of Agricultural Research, and the writer takes this opportunity of recording his indebtedness to the Council. Acknowledgments are also due to the Department of Agriculture, Punjab, for the loan of apparatus and other facilities for work.

frequent shaking till no more bubbles of CO_2 are formed. The soil is then transferred to a filter paper and washed with $0.05N$ HCl till the filtrate shows no reaction for Ca ions; this is followed by leaching with water till the soil is free from Cl ions. It is then washed with a little alcohol and air dried. The final washing with alcohol makes the soil loose and friable as well as easily detachable from the filter paper.

For washing purposes, a Buchner funnel is generally convenient, though a better method is to use the filtering apparatus described by the writer elsewhere (3), with the modification that the cylinder portion need be only 2 or 3 inches long and provided with a lip.

After being air dried the soil is transferred to a flat dish. If a hardened filter paper, like the Watman 50, is used the soil comes off quantitatively, with the help of a spatula and camel's hair brush. The soil is then kept over normal ammonia and subsequently over 90 per cent H_2SO_4 exactly as in the case of natural soil.

The state of saturation of the soil (V) is calculated from the formula

$$(V) = \frac{100\ (T - S)}{T}$$

where S and T are the amounts of ammonia taken up by the natural and acid treated soil respectively. All quantities, of course, are expressed in equivalents

pH value measurements

The greatest difficulty in correlating any physico-chemical measurement on soils with their pH values, lies in the choice of the method for the determination of the latter. The fact that whereas all methods more or less agree among themselves in the case of buffer solutions, they show wide variations in the case of soils is clear evidence that the presence of the soil introduces a variable that is by no means constant or determinable. For the present investigation it appeared necessary to make measurements with all the available methods, in order to see which of them correlates best with the state of saturation of the soil as determined by the ammonia method.

Fifty-seven soils from various parts of India were examined for their reaction by the following methods:

1. Hydrogen electrode 1:5 soil-water ratio, 2 hours' shaking (H).
2. Quinhydrone 1:5 soil-water ratio, 2 hours' shaking (Q).
3. Quinhydrone 1:1 soil-water ratio, 2 hours' shaking (Q′).
4. Quinhydrone 1:1 soil-water ratio, 2 hours' shaking and leaving overnight (Q″).
5. Antimony electrode 1:1 soil-water ratio, 2 hours' shaking and leaving overnight (Sb).
6. Colorimetric method 1:5 soil-water ratio, occasional stirring, and leaving overnight (C). Clear solution obtained by filtering through the percolating cylinder described by the author (3).

The results given in table 1 indicate the magnitude of variations to be expected in the different methods, and as a result of slight alterations in the technique. The relation between (*V*) and various sets of pH values is brought out best by working the correlation coefficients between them. These are given below:

(*V*) and (H) = 0.897
(*V*) and (Sb) = 0.891
(*V*) and (Q'') = 0.889
(*V*) and (C) = 0.938

It will be seen that the correlations are highly significant. The difference is not very great but with the colorimetric method slightly better correlation is obtained than with the other methods. The last column in table 1 gives the pH values calculated from (*V*) values by the following emperical formulas:

1. pH $= 0.0274\,(V) + 5.4$ for (*V*) values below 45 per cent
2. pH $= 0.0319\,(V) + 5.89$ for (*V*) values between 45–81 per cent
3. pH $= 0.0319\,(V) + 7.11$ for (*V*) values above 81 per cent

The difference between the calculated values (C') and those obtained by the colorimetric method, except for soils having (*V*) above 81 per cent, are also recorded in table 1. For the latter, the calculated values are compared with the H electrode, and recorded in the same table. The magnitude of these differences except in the case of half a dozen soils, is not greater than what might be expected from the nature of such measurements, and is of the same order as differences between measurements of pH values by any two methods. The calculated values of pH are therefore probably just as reliable as those obtained by any direct method. It might be mentioned that of the soils that show the greatest discrepancy, P.C. 15 and P.C. 32 contain a large amount of humus. Soils with low *T* values like P.C. 25 are likely to have considerable error in the determination of (*V*).

In working out the correlations, soils having (*V*) above 81 per cent were left out, as these obviously fall in a separate group. Nor can it be expected that a linear relationship will hold good for the whole range, for that would mean that the titration curve of a soil is a straight line, which it is not. Besides, the quinhydrone values for highly saturated soils are not reliable; they are merely recorded for comparison.

Apart from the inherent weakness that a specified technique must be followed for the measurement of pH values, it is a well-known fact that such values when found need careful handling to prove of practical utility. There is, for instance, no significant correlation between pH values and lime requirement of soils. The limit of toleration of plants lies within wide ranges of pH values so that an approximate value is all that is necessary to find. The determination of (*V*) values, on the other hand, will give far more valuable information regarding a soil, at the same time giving an approximate idea of its reaction.

Some very interesting facts emerge from a closer study of the (*V*) values for various soils. It is found that all soils having (*V*) values less than 33.3 per

TABLE 1

The relation between the state of saturation (V) and the pH value of soils

SOIL LABORATORY NUMBER P.C.	LOCALITY	CLAY 0.002 MM.	T (PER 100 GM. SOIL)	(V)	pH (H)	pH (Sb)	pH (Q)	pH (Q')	pH (Q")	pH (C)	pH (C')	(C) – (C')
				per cent								
9	Malabar	26.1	8.1	0	5.76	5.86	5.60	5.47	5.46	6.0	5.4	+0.6
12	Tocklai (Assam)	7.2	4.6	0	5.84	5.50	5.12	4.87	4.93	5.2	5.4	−0.2
14	Madras	21.5	17.2	0	5.37	5.54	5.24	5.16	5.16	5.5	5.4	+0.1
6	Dacca (Bengal)	28.4	11.0	23.6	5.29	5.28	5.16	4.97	4.92	5.1	6.05	−0.95
20	Burma	8.1	4.4	27.3	5.64	6.39	5.58	5.32	5.56	6.4	6.15	+0.25
37	Rangpur (Bengal)	4.1	7.6	30.3	5.75	6.02	5.72	5.50	5.58	6.6	6.23	+0.37
15	Shillong (Assam)	22.4	14.3	31.5	7.71	7.31	7.07	6.74	6.73	7.6	6.26	+1.37
32	Tharavoor (Travencore)	64.6	43.9	31.7	5.05	5.39	5.02	4.73	4.83	5.6	6.27	−0.67
18	Burma	22.6	11.7	33.3	5.79	6.56	5.53	5.32	5.30	6.6	6.31	+0.29
25	United Provinces	4.0	2.1	33.3	7.41	7.52	7.24	7.28	7.14	7.2	6.56	+0.89
22	Burma	15.1	12.7	42.5	6.85	6.56	6.60	6.40	6.46	7.0	7.49	−0.44
19	Mandelay	42.3	24.05	50.1	8.40	7.72	8.28	8.03	7.87	8.0	7.52	+0.51
45	Bihar	11.1	5.8	50.9	7.47	7.87	7.84	7.82	7.64	7.7	7.55	+0.18
49	Madhupur (Bengal)	23.3	17.0	51.8	6.33	6.28	6.49	6.12	6.17	7.0	7.57	−0.55
21	Burma	13.5	12.9	52.7	8.25	7.78	8.01	7.83	7.75	7.95	7.60	+0.38
23	Burma	11.3	11.1	53.6	7.41	7.29	7.29	7.10	7.15	7.5	7.60	−0.10
11	Coimbatore (Madras)	32.8	23.7	54.0	8.77	8.74	8.82	8.64	8.54	8.0	7.62	+0.38
48	Montgomery (Punjab)	19.8	4.7	55.3	8.55	8.48	8.39	8.53	8.29	8.2	7.66	+0.54
46	Baroda (Bombay)	56.4	51.2	56.3	7.63	7.32	7.91	7.42	7.20	7.7	7.69	+0.01
3	Dharwar (Bombay)	62.2	63.1	57.8	7.64	7.14	8.25	7.62	7.29	7.3	7.74	−0.44
44	Bihar	8.4	6.5	59.2	8.54	8.58	8.30	8.43	8.28	7.9	7.78	+0.12
35	Gurgaon (Punjab)	18.3	11.6	59.5	7.98	7.85	8.12	8.06	7.97	7.7	7.79	−0.09
10	Coimbatore	35.6	25.1	60.2	8.71	8.58	8.78	8.54	8.46	8.1	7.81	+0.29
13	Nagpur (C.P.)	58.9	48.0	60.2	8.53	8.05	8.78	8.34	8.23	7.7	7.81	−0.11
34	Gurdaspur (Pb.).	11.3	7.0	60.7	7.63	7.38	7.64	7.49	7.45	7.2	7.83	−0.63
29	Jubbolpur (C.P.).	63.0	49.1	61.1	8.11	7.75	7.72	7.63	7.53	7.9	7.84	+0.06

5	Punjab	12.3	7.32	61.75	8.77	9.12	8.77	8.93	8.88	7.9	7.86	+0.04
42	Bombay	53.4	56.3	62.2	9.00	8.45	8.93	8.47	8.47	8.3	7.88	+0.42
31	Travancore	22.8	17.1	62.6	9.01	7.58	7.83	7.53	7.54	7.7	7.89	−0.19
40	Bombay	13.1	11.0	62.7	7.65	7.48	7.69	7.57	7.51	7.7	7.89	−0.19
43	Bihar	21.6	15.3	62.7	8.41	8.54	8.38	8.31	8.12	8.1	8.20	−0.10
41	Poona (Bombay)	53.4	51.7	63.1	8.74	8.44	8.87	8.60	8.45	8.2	7.91	+0.29
26	Hebbal (Mysore)	22.5	5.7	63.2	8.11	7.90	7.72	7.63	7.53	7.8	7.91	−0.11
50	Ternab (N.W.F.)	17.7	9.6	63.5	8.54	9.00	8.67	8.77	8.71	8.3	7.92	+0.38
38	Broach (Bombay)	52.9	47.3	63.8	8.29	8.21	8.59	8.26	8.30	8.0	7.93	+0.07
8	Coimbatore	25.2	18.7	64.2	8.41	8.42	8.51	8.32	8.31	7.7	7.94	−0.24
17	Kalyanpur (U.P.)	14.1	8.2	64.4	8.20	7.84	8.12	7.99	7.90	8.0	7.95	+0.05
52	Sabour (Bihar)	11.3	6.2	64.5	8.02	8.35	8.01	8.06	7.99	7.6	7.95	−0.35
4	Punjab	15.2	8.2	64.6	8.55	8.66	8.76	8.83	8.77	8.85	7.95	−0.10
16	Lyallpur (Pb.)	8.7	4.9	65.5	8.74	8.38	8.35	8.45	8.33	8.1	7.98	+0.12
1	Pusa (Bihar)	11.3	4.8	66.0	8.41	8.19	8.08	8.13	7.90	8.1	8.00	+0.10
2	Akola (Bombay)	59.3	57.1	66.8	8.21	7.73	8.45	8.15	7.80	8.1	8.02	+0.08
51	Tarnab (N.W.F.)	22.2	7.6	67.1	8.68	8.94	8.62	8.69	8.62	9.0	8.03	+0.97
24	Meerut (U.P.)	9.7	8.8	68.2	8.59	8.69	8.34	8.42	8.30	8.2	8.07	+0.13
27	Mysore	53.2	51.3	69.4	9.03	8.40	9.01	8.74	8.53	8.3	8.11	+0.19
30	Hoshangabad (C.P.)	54.1	57.5	69.9	8.45	8.23	8.74	8.36	8.38	8.1	8.12	−0.02
39	Nadiad (Bombay)	8.4	11.9	72.3	9.11	8.82	8.63	8.71	8.46	8.1	8.20	−0.10
36	Hansi (Punjab)	11.7	6.85	74.5	8.46	8.48	8.33	8.43	8.31	8.1	8.27	−0.17
54	Montgomery (Punjab)	3.2	2.8	78.6	8.71	8.08	8.50	8.54	7.92	8.0	8.40	−0.40
56	Sakrand (Sindh)	13.1	4.9	79.6	8.53	8.72	8.29	8.19	8.10	8.5	8.45	+0.07
55	Sakrand (Sindh)	11.0	7.8	80.8	8.36	8.41	8.12	7.94	7.75	8.1	8.47	−0.37
												(H) − (C′)
47	Montgomery	17.1	6.6	81.8	9.98	9.86	8.76	9.02	8.71	8.7	9.71	+0.27
53	Montgomery	17.6	6.0	85.0	9.87	9.10	9.10	9.04	8.89	....	9.81	+0.06
7	Montgomery	21.8	7.1	90.0	9.58	9.27	8.88	8.86	8.88	9.2	9.97	−0.39
60	United Provinces	7.4	7.3	95.9	10.19	10.35	9.73	9.79	9.89	....	10.16	+0.03
57	United Provinces	4.4	9.0	96.7	10.40	10.20	9.39	9.22	9.02	....	10.19	+0.21
58	United Provinces	13.4	4.7	100.0	10.36	10.78	9.86	9.95	10.13	....	10.29	+0.06

cent respond to lime, the calculated pH value for which is about 7. The neutral point, or pH 7, therefore, has a more fundamental significance than has been hitherto supposed. Most of the good agricultural soils have (*V*) values between 50 and 70 per cent. Soils having (*V*) values above 70 per cent invariably contain exchangeable sodium, and above 80 per cent are all barren alkali soils. Soils that were in approximate equilibrium with $CaCO_3$ under field conditions gave values about 62–68 per cent. All soils having (*V*) values less than 40 per cent are from humid regions where the rainfall is plentiful, and those with above 70 per cent (*V*) values are from arid regions where the rainfall is scarce.

If 33.3 per cent saturation is taken as the basis for lime requirement, the ammonia method will give values comparable with the pH method of Hardy and Lewis (1) for the lime requirement of soils, which consists in bringing the soil to pH 7. If, on the other hand, the values of lime requirement are to be compared with the methods of Hutchinson, or Kappen, which are equivalent to bringing the soil to pH 6.5 (2), the lime requirement should be calculated to 25 per cent (*V*) value. Hissink's method for determining (*V*) values gives the lime requirement at 28 per cent degree of saturation, which is not very different.

The writer has shown elsewhere (5) that the soil acidoid is a tribasic acid containing three equivalents of hydrogen designated as H_1, H_2, H_3. It was also shown that the lime hunger of a soil, from the practical point of view, is satisfied with the neutralization of H_1, which also amounts to 33.3 per cent saturation. It is a remarkable fact that a number of methods of lime requirement of soils should correspond to the same (*V*) value.

The writer had once expressed the view that soils in equilibrium with $CaCO_3$ should be considered as saturated (6), for this appeared to be the limit beyond which any base added was easily hydrolyzable; although it was recognized that the most comprehensive definition of saturation point would be that according to Hissink. In a later investigation certain soils were found that had to be classed as supersaturated (4). The present method, however, avoids that confusion, and it is now possible to include all types of soils under one scheme of classification according to their state of saturation. Incidently, since most of the alkali soils contain bicarbonates, Hissink's method of finding their state of saturation would not be applicable, because a part of $Ba(OH)_2$ will be precipitated and shown as absorbed.

It is evident that any method which aims at giving 100 per cent saturation for soils in equilibrium with $CaCO_3$ will require the soil to be brought to 50 per cent saturation for finding the lime requirement, and the results could be made comparable to those with the ammonia method by a simple calculation.

SUMMARY

Interaction between ammonia and soils, before and after dilute acid treatment, affords a means of characterizing the state of saturation of soils.

From the values of degree of saturation thus obtained, it is possible to calculate the lime requirement as well as the approximate pH value of a soil.

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A NEW METHOD OF DETERMINING CLAY CONTENT OF SOILS BY MOISTURE ABSORPTION AT 70 PER CENT HUMIDITY

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The conception of hygroscopic coefficient, i.e., the percentage of moisture taken up by dry soil from an atmosphere saturated with water vapors, is an old one; and at one time was regarded as a very important constant for characterizing a particular soil. The interest in it received a fresh impetus from the researches of Briggs and Shantz, who gave empirical formulas correlating hygroscopic coefficient with wilting coefficient as well as the mechanical composition of soils (1). It was soon recognized, however, that these empirical formulas had only a limited applicability, and only a rough correlation between mechanical composition and hygroscopicity was all that could be reasonably expected.

The difficulty in finding a more rigid relationship lay in the very nature of things. Methods of determining clay (which is mainly responsible for moisture absorption) as well as hygroscopic coefficient were extremely faulty. With the introduction of newer methods of mechanical analysis of soils clay can be determined with great precision; but hygroscopic coefficient still remains a factor easy to imagine theoretically, but most difficult to attain in practice, even when the technique is refined considerably beyond that reasonably possible in routine determinations (3).

The difficulty of maintaining a saturated atmosphere for any length of time led Mitscherlich to suggest (2) that hygroscopicity of the soil should be determined by bringing it into equilibrium with the partial pressure of water vapor afforded by 10 per cent H_2SO_4 (i.e. 96 per cent humidity). Somewhat similar considerations led the U. S. Bureau of Soils workers to recommend 3.3 per cent H_2SO_4 (i.e. 99 per cent humidity), for the same purpose. It must be admitted, however, that these concentrations are fixed arbitrarily, and there is no valid ground for the belief that they represent any fundamental advance on the original conception of the hygroscopic coefficient.

The first important step forward in our knowledge regarding hygroscopicity of soils is due to Thomas (9) who measured the vapor pressure of soils at differ-

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[1] This work was brought to completion by the help of a grant from the Imperial Council of Agricultural Research, and the writer takes this opportunity of recording his indebtedness to the council. Acknowledgments are also due to the Department of Agriculture, Punjab, for the loan of apparatus and other facilities for work.

ent moisture contents. This was followed by the work of Puri et al. (8) on the relation between vapor pressure and moisture content of soils, in which the suggestion was put forward that hygroscopicity of soils should be measured by bringing it into equilibrium with an atmosphere of 50 per cent humidity, as this occurred on the point of inflection of vapor pressure-moisture content curve and any error in controlling vapor pressure would only result in a small difference in equilibrium moisture content.

Another difficulty about any moisture absorption experiment is the definition of "dry" soil. The usual practice is to dry in an oven at 100–105°C. This has the advantage of rapidity but little confidence can be placed in it. Drying over H_2SO_4 is to be preferred, though the process is slow. Both of these methods are frequently attended with permanent changes in the soil colloids. In order to get over this difficulty the author has suggested elsewhere (6) that since the interval between 10 per cent and 70 per cent humidity on a vapor pressure curve for soil is sensibly straight (fig. 1), this should be made the basis of expressing hygroscopicity of soils. It was also shown that the influence of exchangeable ions in soil colloids is felt the least in this portion of the curve and by adopting it as a "single value" we shall be expressing the specific surface of the soil particles, irrespective of the nature and extent of the extraneous influences that generally come into play in soils.

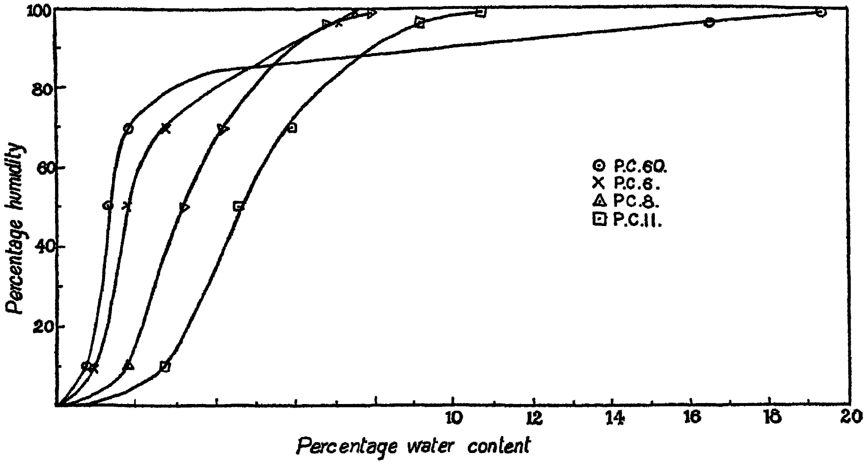

FIG. 1. TYPICAL VAPOR PRESSURE CURVES OF TWO NORMAL SOILS (P.C. 8 and 11), A HIGHLY ACID SOIL (P.C. 6), AND A HIGHLY ALKALINE SOIL (P.C. 60)

A great feature of the vapor pressure curve of soils is that normal soils generally give normal curves, whereas abnormal curves are almost invariably obtained with highly acid or highly alkaline (especially those containing exchangeable Na or free Na_2CO_3) soils (fig. 1), but for all soils the portion between 10–70 per cent humidity is the least affected.

It will be clear from the nature of the curves shown in figure 1 that the hitherto considered "dry point" is quite as indefinite as the equilibrium point with saturated atmosphere. By adopting 10 per cent humidity equilibrium value as the "dry" basis, we can be sure of a greater precision in the determination of this reference point. On this basis the vapor pressure curve up to 70 per cent humidity being a straight line passing through the origin, the determination of a single value will enable us to interpolate hygroscopicity at any other humidity between 10 and 70 per cent.

Since hygroscopicity (10–70 per cent humidity) is only slightly affected by the nature of soil colloids, it should show a better correlation with clay content in different soils. This is actually the case. Indeed it is possible to calculate the percentage of clay (0.001 mm. diameter) in a given soil by determining its hygroscopicity in this manner (table 1).

It is to be noted that the hygroscopicity values for a given humidity are higher when that humidity has been reached by drying than when it is reached by wetting (8). Both the values were determined for the 57 soils examined. The correlation coefficient when the equilibrium values were reached by drying was found to be 0.996, and when they were reached by wetting the correlation was 0.993. Both these values are highly significant. The following relations hold good between clay (0.001 mm.) and hygroscopicity, (H).

Drying

$$\text{Clay} = 8.04\,H + 1.02$$

Wetting

$$\text{Clay} = 8.41\,H + 2.8$$

In table 1 the calculated values of clay are the mean of the drying and wetting hygroscopicity values. The agreement between the calculated values and those found by direct determination with the pipette method, using the NaCl-NaOH method of dispersion described by the author elsewhere (5), is as good as could be expected from a highly complex body like soil, when the errors involved in the measurements are considered. The few soils that show rather serious discrepancy are invariably abnormal soils. Soil P.C. 6 is a highly acid soil entirely barren, P.C. soils 53, 56, 57, 58 and 60 are highly alkaline barren soils containing free Na_2CO_3.

Absorption of moisture from humidities higher than 70 per cent is largely affected by the state of aggregation of the soil colloids. It would appear therefore that the amount of moisture absorbed by a soil between 70–99 per cent humidity will indicate characteristic differences between soil colloids from various sources depending on their state of aggregation. However, to reduce such moisture absorption to a common denominator in all soils the water thus absorbed should be divided by hygroscopicity (10–70 per cent humidity) which has been shown to be a function of the total clay content. Table 1 also includes these values for 58 soils. The various soils have been arranged according to the province from which they were obtained. It will be seen that on the whole soils from any particular province show approximately the same (70–

TABLE 1

*The relation between hygroscopicity, clay content, and dispersion coefficient**

	SOIL	H	CLAY (0.001 MM.)		DIFFERENCE	$\frac{H'}{H}$	D.C.
			Calculated	Found			
		per cent	*per cent*	*per cent*	*per cent*		*per cent*
Punjab soils	P.C. 4	0.79	8.4	10.5	+2.1	2.96	23.7
	P.C. 5	0.56	6.5	9.6	+3.1	3.23	30.8
	P.C. 7	1.95	17.9	17.5	−0.4	7.48	94.7
	P.C. 16	0.52	6.1	5.4	−0.7	4.02	20.7
	P.C. 34	0.68	7.4	8.1	+0.7	2.40	7.1
	P.C. 35	1.75	16.3	16.9	+0.6	0.90	4.9
	P.C. 36	0.74	8.0	9.0	+1.0	2.39	5.1
	P.C. 47	1.01	10.2	12.8	+2.6	3.49	57.9
	P.C. 48	1.10	10.9	15.7	+4.8	3.68	9.6
	P.C. 53	0.85	8.8	15.2	+6.4	3.36	61.3
	P.C. 54	0.15	3.1	2.6	−0.5	4.67	20.2
U. P. soils	P.C. 17	1.00	10.1	8.9	−1.2	3.84	8.5
	P.C. 24	0.83	8.7	5.5	−3.2	3.28	4.5
	P.C. 57	0.60	6.8	1.9	−4.9	3.10	49.4
	P.C. 58	2.12	19.3	11.7	−7.6	7.72	100
	P.C. 59	0.81	8.6	7.8	−0.8	1.93	26.5
	P.C. 60	1.24	12.2	4.8	−7.4	14.40	92.3
C. P. soils	P.C. 13	6.32	53.9	56.5	+2.6	1.28	6.4
	P.C. 28	4.35	37.7	42.8	+5.1	1.22	1.8
	P.C. 29	6.34	54.0	61.1	+7.1	1.31	2.9
	P.C. 30	6.72	57.1	53.5	−3.6	0.96	2.7
Bombay soils	P.C. 2	6.04	51.6	56.1	+4.5	1.33	2.7
	P.C. 3	6.69	56.7	61.2	+4.5	1.16	3.7
	P.C. 38	5.59	47.8	50.9	+3.1	1.17	2.3
	P.C. 39	1.30	12.5	7.2	−5.3	1.44	5.9
	P.C. 40	1.26	12.3	11.6	−0.7	1.52	2.3
	P.C. 41	6.39	54.4	51.6	−2.8	1.11	2.4
	P.C. 42	7.01	59.5	54.4	−5.1	1.28	2.2
	P.C. 46	6.58	56.0	55.9	−0.1	1.27	1.6
Bihar soils	P.C. 1	0.31	4.5	6.4	+1.9	5.52	26.5
	P.C. 43	1.90	17.5	16.2	−1.3	3.07	7.4
	P.C. 44	0.37	5.0	5.4	+0.4	4.68	5.9
	P.C. 45	0.78	8.3	8.9	+0.5	2.24	5.4
	P.C. 52	0.82	8.6	10.5	+0.9	2.37	6.2
Bengal soils	P.C. 6	1.79	16.6	25.2	+8.6	2.87	4.2
	P.C. 37	0.45	5.6	2.8	−2.8	2.47	22.9
	P.C. 49	2.73	24.3	26.7	+2.4	1.68	2.6

TABLE 1—*Concluded*

	SOIL	H	CLAY (0.001 MM.)		DIFFERENCE	$\frac{H'}{H}$	D.C.
			Calculated	Found			
		per cent	*per cent*	*per cent*	*per cent*		*per cent*
Madras soils	P.C. 8	2.59	23.2	22.5	−0.7	1.42	4.0
	P.C. 9	1.51	14.3	15.9	+1.6	4.13	4.6
	P.C. 10	3.79	33.0	32.2	−0.8	1.50	2.8
	P.C. 11	3.26	28.6	29.3	+0.7	1.49	3.2
	P.C. 14	2.51	22.5	16.6	−5.9	2.98	27.9
Assam soils	P.C. 12	0.60	6.8	8.8	+2.0	9.62	9.7
	P.C. 15	1.92	17.6	17.2	−0.4	4.13	5.1
Burma soils	P.C. 18	1.35	13.2	15.1	+1.9	2.98	17.1
	P.C. 20	0.34	4.7	5.3	+0.6	5.15	8.6
	P.C. 21	1.33	12.8	9.7	−3.1	2.42	8.6
	P.C. 22	1.02	10.3	11.9	+1.6	2.55	5.3
	P.C. 23	0.98	9.5	8.9	−0.6	2.18	6.4
Travancore soils	P.C. 31	2.17	19.7	20.9	+1.2	1.64	1.3
	P.C. 32	6.81	57.7	56.3	−1.4	3.07	3.4
Mysore soils	P.C. 26	3.11	27.4	22.4	−5.0	0.89	3.1
	P.C. 27	6.35	54.1	52.6	−1.5	1.17	2.3
Sind soils	P.C. 55	0.96	9.8	6.8	−3.0	3.98	15.3
	P.C. 56	1.37	13.2	8.7	−4.5	5.93	20.6
N. W. F. soils	P.C. 50	1.47	13.9	13.8	−0.1	2.55	11.3
	P.C. 51	1.08	10.7	9.7	−1.0	2.76	14.7

* H = hygroscopicity between 10–70 per cent humidity.
H′ = hygroscopicity between 70–99 per cent humidity.
D. C. = Dispersion coefficient.

99 per cent) hygroscopicity per unit surface area with here and there abnormally high values the significance of which will be understood from what follows:

Moisture from the vapor phase may be absorbed by soils in three ways. It may enter into loose chemical combination and exist as water of hydration; it may be taken up in the minute capillaries on the surface of individual particles (2); or it may fill up the interstitial spaces between the aggregates of various particles. It is conceivable that the portion of the vapor pressure curve below 10 per cent humidity (fig. 1) represents water of hydration; between 10–70 per cent humidity, capillary adsorbed water and the portion above interstitial water. Water of hydration will depend on the constitutional nature of soil colloids, the capillary absorbed water on the extent of their surface area, and interstitial water on their state of aggregation. The correctness of this hypothesis in the case of capillary absorbed water is shown by the fact that this moisture is correlated with the total clay content. In the case of interstitial water it is supported by the fact that we have an independent method of determining the state of aggregation of the soil particles, i.e., the measurement of dispersion coefficient (7), and as is seen from table 1, soils in general show a positive correlation between interstitial water per unit surface and dispersion coefficient. The correlation coefficient works out to be 0.765, which is highly significant. The method of determining dispersion coefficient may be briefly stated here:

Ten grams of soil is made up to one liter with water and left overnight in a cylinder or bottle of uniform diameter, after the soil and water have been mixed by turning the bottle end over end a few times, taking care that the suspension is not subjected to any violent agitation which might break up the crumbs. Next morning the suspension is again thoroughly mixed by gentle end over end motion and allowed to settle for the appropriate time, and clay (0.002 mm.) is determined by pipetting from the proper depth exactly as in the pipette method of mechanical analysis. The clay thus obtained is called the dispersion factor (D.F.). The total clay (C) obtainable on complete dispersion of the soil is determined by the author's NaCl-NaOH method (5). Dispersion coefficient (D.C.) is calculated by the formula

$$\text{D C.} = \frac{\text{D F.} \times 100}{\text{C}}$$

A sharp distinction between the three forms of hygroscopic moisture is not possible by the very nature of things. For one thing there are no sharp breaks in the vapor pressure curve; but from an examination of these curves it may be safely stated that fixing 10–70 per cent humidity limits is not altogether arbitrary, and will eventually help us in elucidating the mechanism of moisture absorption much better than the simpler conception of the hygroscopic moisture as a uniform film of water round the soil particles.

SUMMARY

The vapor pressure curve for soils could be split up into three portions representing three forms of hygroscopic moisture i.e. hydration, capillary absorbed, and interstitial. The first depends on the constitutional nature of soil colloids, the second on their surface area, the third on their state of aggregation.

Though a sharp distinction could not be made between the three forms of moisture, there is evidence to show that we shall not be far wrong in considering 10 per cent humidity as marking the end of the first form, and 70 per cent humidity as the end of second form.

Clay (0.001 mm.) content of a soil can be estimated from moisture absorption between 10–70 per cent humidity with a fair amount of accuracy, by the help of an empirical formula.

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IRON IN RELATION TO THE STIMULATION OF GROWTH BY HUMIC ACID[1]

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Humic acid stimulates the rates of growth of various higher and lower plants. Many attempts have been made to determine the cause of its beneficial action. Kaserer (25, 26) suggested a connection with rare inorganic nutrients such as aluminum or silica added with the humic acid; Söhngen (47) a connection with colloidal absorption and consequently greater availability of nitrogen and oxygen gases; Allen (1) with phosphate nutrition; Bortels (5) with molybdenum stimulation; Bottomley (6) and Mockeridge (34, 35) with "organic accessory food substance" or "auximone" nutrition; and Olsen (38), Remy and Rösing (39), Söhngen (47), Kaserer (25), Fred (19), and Koch et al. (28) with iron nutrition. Greaves (22) also suggested a protective action against poisons, such as arsenic, by colloidal absorption. It has often been suggested that humic acid increases the availability of one or more of the known constituents already present in the medium, rather than that it adds some stimulant or stimulants to the culture medium. At the outset of the present studies the writers were inclined to believe that probably some one of the numerous organic constituents of humic acid was specifically responsible for the stimulating effect.

It is the purpose of this paper to present evidence favoring the view that humic acid acts primarily by virtue of its iron component. The free living, aerobic soil organism *Azotobacter vinelandii* will receive chief consideration in respect of the studies on growth stimulation.

EXPERIMENTAL PROCEDURE

Preparation of humic acid

Humic acid is not a single homogeneous compound. It consists chemically of a large number of different compounds which may be extracted from soil organic matter or prepared synthetically from sugars, or other similar organic materials. In this paper humic acid will be considered as consisting of any of those compounds which may be *reversibly dissolved in dilute alkali and precipitated in dilute acid* (see also footnote 3). The chemical constitution

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[1] A brief note relating to the work in this and the following paper appeared in *Science* (1931) 74: 522–4, and can be read simultaneously with advantage.

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TABLE 1

*Description of humic acids employed**

HUMIC ACID	SOURCE	DATE OF PREPARATION	NUMBER OF PURIFICATIONS§	METAL ADDED** PER 100 GM. SUGAR	DIGESTION	PER CENT Fe††
				gm.		*per cent*
(a) Natural						
I†	Soil A (garden)	VIII/-/29	4	....	....	(0.68)§§
II	Soil A	VII/-/30	4	....	....	0.68
III	Soil B (poor soil)	VIII/-/30	4‖	....	....	0.06‡‡
IV	Soil B	VIII/-/30	4	....	....	0.11
V	Soil C (rich soil	VIII/-/30	4	....	....	1.4
(b) Synthetic‡						
VI	50 g. glucose, 250 cc. 35 per cent HCl	X/-/30	4	....	Boiling water bath for 2 hours	0.03‡‡
VII	50 g. glucose, 250 cc. 35 per cent HCl	X/-/30	6-10	Fe 1.0		0.30
VIII	50 g. glucose, 250 cc. 35 per cent HCl	II/-/31	6	....	72° for 15 hours	0.03‡‡
IX	50 g. glucose, 250 cc. 35 per cent HCl	II/-/31	11	Fe 4.0		4.4
X	50 g. glucose, 250 cc. 35 per cent HCl	II/-/31	13	Fe 4.0	Slowly boiled over flame for 15 minutes	3.2
XI	50 g. glucose, 250 cc. 35 per cent HCl	II/-/31	2	Fe 4.0		4.4
XII¶	50 g. glucose, 250 cc. 35 per cent HCl	V/-/31	5	Mo 2.5		0.03‡‡
XIII¶	50 g. glucose, 250 cc. 35 per cent HCl	V/-/31	5	Al 2.5		0.03‡‡
XIV¶	50 g. glucose, 250 cc. 35 per cent HCl	V/-/31	5	P 2.5		0.03‡‡
XV¶	50 g. glucose, 250 cc. 35 per cent HCl	V/-/31	5	Si 2.5		0.03‡‡
XVI¶	50 g. glucose, 250 cc. 35 per cent HCl	V/-/31	5	Co 2.5		0.03‡‡
XVII¶	50 g. glucose, 250 cc. 35 per cent HCl	V/-/31	5	Mn 2.5		0.03‡‡
XVIII¶	50 g. glucose, 250 cc. 35 per cent HCl	V/-/31	5	....		0.03‡‡
XIX¶	50 g. glucose, 250 cc. 35 per cent HCl¶¶	VII/-/31	4	....		....

* Throughout this paper the various humic acids will often be referred to in abbreviated form; thus Humic Acid I = H. A.-I, etc. Although the acids are employed in experiments in the form of their potassium salts, properties such as concentration and constitution will refer to the acids themselves.

† H. A.-I contained 4.3 per cent nitrogen as shown by Kjeldahl analysis. No other analyses of nitrogen in the different natural humic acids were made, but this analysis is probably representative.

‡ After the initial process of acid digestion the mixture, containing solid humic acid and charred sugar, was centrifuged (liquid discarded) and the humic acid in the precipitate extracted and purified as in the case of the natural humic acids.

§ As described in the text, each purification consisted of solution by alkali, reprecipitation with acid, centrifugation, and decantation.

¶ H. A.-XII to XIX were filtered instead of centrifuged.

‖ Washed with HCl for an unusually long time (16 hours), rather than about 1 hour, as in the case of the other natural humic acids.

** Added as a soluble salt at start of digestion, Fe as $Fe_2(SO_4)_3$, Mo as MoO_3, Al as $Al_2(SO_4)_3$, P as K_2HPO_4, Si as Na_2SiO_3, Co as $CoSO_4$, and Mn as $MnCl_2$.

†† Iron was analyzed by the colorimetric thiocyanate method of Scott (45), the sample of humic acid of known dry weight first being ignited and taken up in acid solution.

‡‡ The error with these relatively small quantities of iron (about 0.03 per cent) may be as great as ±0.02 per cent.

§§ No iron analysis was made in the case of H. A.-I, but the latter was prepared in the same manner, and from the same soil, as H. A.-II, and was identical physiologically (see table 4D).

¶¶ The glucose employed to prepare H.A.-XIX was first treated to remove, so far as possible, the trace of iron impurity (0.00045 per cent) existing, by allowing a 20 per cent solution of glucose to stand for 1 hour at pH 10 and then filtering. Analyses indicated that a large part of the iron impurity in the glucose was removed by this treatment. Most of the iron present in H. A.-VI, VIII, and XII–XIX was undoubtedly derived from the HCl employed in their preparation.

of most of the individual organic compounds contained in humic acid is as yet unknown, although several of high molecular weight have been definitely identified (Schreiner and Shorey, 44, 44a, 44b, 46a, 46b). Natural soil humic acids contain generally nitrogen and various inorganic elements also. Thus Remy and Rosing (39) found that their carefully prepared active sample of soil humic acid contained 2.4 per cent nitrogen, 1.84 per cent Fe_2O_3, 3.12 per cent Al_2O_3, 6.9 per cent SiO_2, and 0.49 per cent P_2O_5. Russell (41, p. 165–178), Mattson (31), Waksman and Reusser (51), and Sven-Oden (50) present reviews of the present state of knowledge concerning the physical and organic chemistry of humic acid.

The natural humic acids used by the writers show an intensive growth-stimulative action with Azotobacter and Rhizobium, when these organisms are grown in artificial media. Remy and Rösing (39), Mockeridge (34), Olsen (38), and other writers have indicated that similarly prepared humic acids stimulate bacterial peptone decomposition, urea decomposition, alcoholic fermentation by yeasts, nitrification, denitrification, growth of legume nodule bacteria, and the growth of higher and lower green plants such as sunflower and duckweed, when grown under natural conditions.

A natural humic acid is prepared as follows. A 100-gm. sample of soil is washed twice with 100-cc. portions of *N* HCl, leached for 12 hours with 200 cc. 10 per cent KOH, and clarified by centrifugation. The decanted, centrifuged solution, containing the humic acid as soluble potassium humate, is reprecipitated by bringing the pH to 3.0–4.0 with HCl, and reclarifying by centrifugation. The precipitate, humic acid, is redissolved and reprecipitated generally about four times in all, and the final precipitate dissolved cautiously in aqueous KOH to give a slightly buffered solution at pH 7.5 containing about 10 mgm. humic acid and 0.4 mgm. nitrogen per cubic centimeter. It is then ready for use in physiological experiments, generally being added, with appropriate dilution, as 25–50 p.p.m. (= 25–50 mgm. per liter) calculated as humic acid. Azotobacter is unable to use either the carbon or the nitrogen in humic acid for nutrition (7, 8, 24, 29). The preparation of synthetic humic acids from glucose when different from that of natural ones, is described in table 1.

Cultural methods

The general methods of culture and analysis employed have been fully described elsewhere (7, 8, 9, 10) and will be but briefly reviewed here. Both the *Warburg* micro-respiration and *Erlenmeyer* flask techniques have been employed.

Warburg technique. The rate of oxygen consumption has been measured according to the physico-chemical, manometric, micro-methods worked out by Otto Warburg (52) for studying cell metabolism. The chief apparatus employed in connection therewith is illustrated in figure 1 and plate 1. The increase in rate of oxygen consumption with time gives a measure of the rate of growth, and is accompanied by a corresponding increase in the number of organisms, or, similarly, the dry weight, turbidity, and cell nitrogen. The manometers can be read frequently, and time curves of the increases of growth determined over very short periods of time, i.e., hours or minutes.

If desired, turbidimetric measurements of the actual growths in the *Warburg* vessels can be made at the end of an experiment, as a check on the indirect indications given by the respiration rate increases.

Unless otherwise indicated the temperature of the *Warburg* technique experiments has been 28 ±0.01°C.

Erlenmeyer flask technique. The cultures have been grown chiefly in 25 cc. of liquid medium contained in 250-cc. *Erlenmeyer* flasks plugged with cotton. In preparing the flasks for sterilization, an additional 10 to 20 per cent by volume of distilled water has been added to make up for losses during sterilization. Sterilization has been performed in an autoclave maintained at 120°C. for 15 minutes. Inoculations have generally been made with one drop of a young culture, so that the inoculum constituted about 0.25 per cent by volume of the total culture. At times, also, the inoculum consisted of one drop of 1 cc. of culture diluted

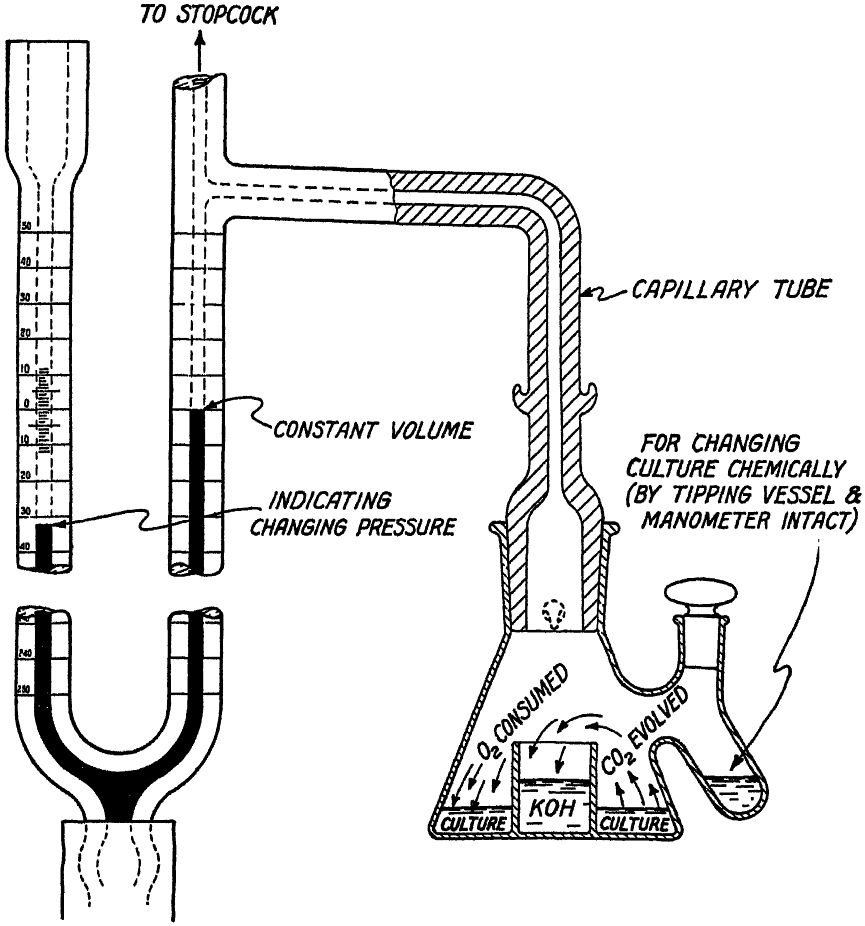

Fig. 1. Diagram of Manometer and Vessel Employed in Measuring Oxygen Consumption and Growth

The total volume of the vessel measured to the right-hand level of the manometric fluid (Brodie solution) is about 16 cc., 2 cc. of which is occupied by the culture and 0.30 cc. by the $2N$ KOH. The remaining volume is accounted for by a gas, usually air, at atmospheric pressure. The right-hand level of the manometric fluid is held constant; the CO_2 evolved in respiration is absorbed in the alkali; the oxygen consumption causes a slight decrease in internal pressure which is registered by a fall in the left-hand level of the manometric fluid; the reading thus obtained, when multiplied by a constant characteristic of the vessel, gives directly the number of cubic millimeters of oxygen consumed, with a usual accuracy of ±0.5 cmm. The vessel constant varies from about 1 to 2, depending upon the temperature, volumes of gas and culture medium, the Bunsen absorption coefficient of the gas concerned, and the normal pressure of the manometric fluid. Under ordinary atmospheric conditions the amount of nitrogen consumption by *Azotobacter* is less than 1 per cent of the simultaneous oxygen consumption and may be neglected. If it is desired to change the chemical environment of the organism during the course of an experimental run, by addition of humic acid, nitrate, etc., a 0.10-cc. portion of an aqueous solution of the chemical, made up to 20 times the desired concentration, and is placed in the side-cup initially and then tipped in when desired.

with 10 cc. sterile distilled water, yielding a culture inoculum 0.025 per cent that of the total culture. The cultures have been maintained in a large air incubator thermostat at 28 ±1°C. Care has been taken in each series of experiments to maintain constant all intended invariant conditions, such as volume, amount of inoculum, temperature, and particularly pH. The initial and final pH values of each culture have been determined colorimetrically, with one or more indicators and *La Motte* standards, and due allowance for pH differences has been made in interpretations of results. The rate of growth, as well as the rate of respiration, is slightly increased as the pH rises from 6.0 to 7.2. Ordinarily the experiments were run in duplicate or in triplicate. The cultures used to inoculate the *Erlenmeyer* flasks, as well as those used in the *Warburg* experiments, were grown in an air thermostat at 28 ±1°C. for 24 to 48 hours, in sterilized, aerated 250-cc. gas wash-bottles containing 50 to 100 cc. of culture medium. The aeration was obtained by means of compressed air passed through the bottles at the rate of about 100 cc. per minute.

Media of varying inorganic constitution have been employed. "Customary" medium contained the inorganic constituents remaining in the clear liquid obtained after the mixture "0.8 gm. K_2HPO_4, 0.2 gm. KH_2PO_4, 0.2 gm. $MgSO_4 \cdot 7H_2O$, 0.2 gm. NaCl, 0.1 gm. $CaSO_4 \cdot 2H_2O$, 0.01 gm. $Fe_2(SO_4)_3 \cdot 9H_2O$, 1,000 gm. H_2O" has been thoroughly shaken, allowed to stand and settle. The term "customary" concentration of salt or ion used throughout this paper will refer, however, to the concentration of that salt or ion obtaining in customary medium before the latter is allowed to settle. Chemical analysis shows that the clear solution contains about 15 per cent less phosphate, 40 per cent less calcium, and 99 per cent less iron than the unsettled solution. Unless otherwise stated, customary medium will always have been employed.

The inorganic constituents of "bicarbonate medium" consisted solely of 0.1 per cent $KHCO_3$.

When iron has been added in the form of citrate, tartrate, oxalate, or sulfate it has been reported in the tables upon the basis of 20 per cent composition, for simplicity, although the actual composition values range from 20–24 per cent.

When fixed nitrogen was present it was added at an initial concentration of 50 p.p.m. N, unless otherwise mentioned. This concentration of fixed nitrogen has been shown (7, 8) to prevent effectively all fixation of nitrogen in short time experiments of 2 days or less, and to place growth dependent entirely upon fixed nitrogen supply, even when nitrogen gas, as in air, is present. Recrystallized KNO_3 was used chiefly.

Although the inorganic constituents of the medium have been varied, the initial glucose concentration has been kept constant at 1 per cent in all experiments, unless otherwise mentioned, and during growth the concentration has rarely decreased below 0.2 to 0.5 per cent. The glucose used in these experiments (*Mallinckrodt, C. P.*) was reported by label to be quite pure, yielding, at a concentration of 1 per cent, a maximum total ash impurity in the cultures of 5 p.p.m. Actual analysis, made by a slightly modified thiocyanate method of Scott (45), showed that the iron fraction of the ash constituted only 0.045 p.p.m. This concentration of iron, however, was considerably larger, even, than that obtaining in clear settled glucose-free customary medium, namely, 0.012 p.p.m. Fe.

Growth has been measured turbidimetrically with a *Bausch and Lomb* nephelometer, after periods of incubation of ordinarily 36 to 60 hours. When the amounts of growth were very small, counting with a haemacytometer was also resorted to. In making the measurements it was always necessary to maintain uninoculated controls, since the amounts of precipitate were variable, and often quite appreciable. When appreciable amounts of turbidity occurred in the uninoculated controls, as would be caused by the presence of precipitated calcium phosphate, iron phosphate, etc., or when the pH of the inoculated culture was different from that of the control, it was often found expedient to acidify inoculated and uninoculated flasks to a constant pH of 3.5, i.e., just acid to brom-phenol blue, in order to dissolve the inorganic precipitates and to leave growth as the only cause of turbidity. Such acidification did not affect the turbidity caused by the organisms. The inoculum itself ordinarily did not change the initial turbidity, but in some cases where growth was small or negligible, control corrections were necessary. At very high concentrations the color of humic acid necessitated a special correction in addition to that caused by the very slight turbidity of humic acid.

Growth has sometimes been measured in the *Erlenmeyer* technique by determining the final rate of respiration. Aliquot samples have been removed from the *Erlenmeyer* flasks and the respiration rates measured in *Warburg* vessels. This combined technique will be termed the "*Erlenmeyer-Warburg*" technique.

By "humic acid ratio" is meant the ratio of the final numbers, or likewise final respiration rates, turbidities, etc., of organisms in any given medium in the presence and absence of humic acid, or a substitute stimulator, after any given period of time, in our experiments usually 2 days but occasionally only a few hours or even a number of days. As will soon be evident, this ratio varies generally from 2 to 4, but may at times be either somewhat higher or lower, depending chiefly upon the *duration* of experiment, the concentration of humic acid, and the actual rate of growth in the absence of humic acid. The ratio will be calculated to two decimal points but no implication of this degree of accuracy shall be intended.

In comparing experiments performed by the *Warburg* and *Erlenmeyer* techniques it is to be borne in mind that in general the velocity of growth is greater in the case of the former because of agitation and other similar factors which permit of more constant and favorable conditions.

SUBSTITUTION OF NATURAL HUMIC ACIDS BY IRON COMPOUNDS AND SYNTHETICALLY PREPARED IRON-CONTAINING HUMIC ACIDS

Tables 2–8, inclusive, show that natural humic acids may be substituted by various organic or inorganic compounds of iron, such as ferric citrate, oxalate, tartrate, and sulfate, or metallic iron, under a wide variety of experimental conditions, as follow: (*a*) technique (*Warburg*, *Erlenmeyer*, *Erlenmeyer-Warburg*); (*b*) medium (customary and bicarbonate); (*c*) duration of experiment (18 hours to 6 days, including 25, 36, 40, 41, 48, 67, and 96 hours); (*d*) criterion of growth (turbidity, respiration rate, Kjeldahl-nitrogen content); (*i*) preparation of natural humic acids (Nos. I, II, V); (*f*) concentration of humic acids and iron compounds; (*g*) final pH (6.0–7.6); (*h*) age of inoculating culture; and (*i*) source of nitrogen [free N_2, and fixed nitrogen such as urea-N (500 and 200 p.p.m.) and NO_3-N (200 and 50 p.p.m.)]. Tables 10, 10A, 11, 11A, 13, and 14B also show humic acid substitution by various iron compounds and under an increased variety of experimental conditions.

Tables 9–15A, inclusive, show that synthetically prepared humic acid[3]

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[3] The synthetic humic acids we have prepared (both the physiologically active and inactive ones) are quite similar to the natural humic acids in the important characterizing properties: (*a*) they are precipitated in mildly acid solutions (pH 3–5); (*b*) soluble alkali salts are easily formed; and (*c*) they are deeply colored, black, or dark brown (depending upon the concentration). In fact, we have observed no significant chemical differences. It is of no great interest here as to how exactly natural and synthetic humic acids are of the same chemical constitution, so long as, possessing many identical chemical properties, the same physiological functions are obtained. Eller and Koch (17) have prepared synthetic humic acids by various methods and have shown them to be similar to natural humic acids with respect to composition, solubility in water, alcohol, and ether, salt formation, color, and chemical reactions with Cl_2, HNO_3, Br_2, acetone, etc. Kawamura (27) has shown that the adsorption-concentration functions with respect to various bases are substantially identical for natural peat, and synthetic cane sugar, humic acids.

TABLE 2

Humic acid substitution by organic and inorganic iron compounds and non-substitution by various other metallic compounds (Erlenmeyer-Warburg technique)

SUBSTANCE ADDED	CONCENTRATION OF METAL	FINAL RESPIRATION RATE* (CMM. O_2 CC. HOUR) (RELATIVE GROWTH)	H. A.-RATIO
None	....	62, 65, 67 56, 62, 59 } 62	....
H. A.-V (50 p.p.m.)	0.7 p.p.m. Fe	146, 167 } 157	2.53
Fe-citrate (10 p.p.m.)	2 p.p.m. Fe	100, 97 } 99	1.60
Fe-tartrate (10 p.p.m.)	2 p.p.m. Fe	103, 101 } 102	1.65
$Fe_2(SO_4)_3$	4 p.p.m. Fe	150	2.42
	3 p.p.m. Fe	187	3.02
	1 p.p.m. Fe	138	2.22
	0.6 p.p.m. Fe	107	1.73
	0.2 p.p.m. Fe	83	1.34
Fe-powder	10 p.p.m. Fe	91	1.47
	0.1 p.p.m. Fe	97	1.56
$CoSO_4$	10 p.p.m. Co	6	About 1.0 or less
	1 p.p.m. Co	68	About 1.0 or less
	0.1 p.p.m. Co	69	About 1.0 or less
$MnCl_2$	10 p.p.m. Mn	55	About 1.0 or less
	1 p.p.m. Mn	56	About 1.0 or less
	0.1 p.p.m. Mn	47	About 1.0 or less
$Al_2(SO_4)_3$	10 p.p.m. Al	66	About 1.0 or less
	1 p.p.m. Al	58	About 1.0 or less
	0.1 p.p.m. Al	65	About 1.0 or less
$NiCl_2$	10 p.p.m. Ni	0	About 1.0 or less
	1 p.p.m. Ni	40	About 1.0 or less
	0.1 p.p.m. Ni	79	About 1.0 or less
$ZnCl_2$	10 p.p.m. Zn	0	About 1.0 or less
	1 p.p.m. Zn	61	About 1.0 or less
	0.1 p.p.m. Zn	68	About 1.0 or less
$Cr_2(SO_4)_3$	10 p.p.m. Cr	19	About 1.0 or less
	1 p.p.m. Cr	54	About 1.0 or less
	0.1 p.p.m. Cr	53	About 1.0 or less
Na_2MoO_4	1 p.p.m. Mo	73	About 1.0 or less
	0.1 p.p.m. Mo	72	About 1.0 or less

Initial culture 1-day old *A. vinelandii*. Initial respiration rate 53 cmm. O_2/cc./hour. Analyzed after 40 hours. Final pH 6.8 ± 0.1 except 10 p.p.m. Al, Co, Ni, Zn, Cr, 6.4 ± 0.1. Source of nitrogen: N_2. V/19–21/31.

* The cultures were grown in *Erlenmeyer* flasks (28°C.) and the final relative growths measured as respiration rates (31°C.)

containing considerable fractions of iron was highly active physiologically, whereas when the iron fraction was negligible, or substituted by various other metals, as in tables 14, 14A, 14B, the physiological action was relatively negligible. The latter observation confirms the general finding [Krzemieniewsky (29), Warmbold (53), Löhnis and Pillai (30), Remy and Rösing (39), and Mockeridge (34)] that synthetic humic acids prepared from sugars are physiologically inactive or substantially so, since, upon looking up the previous work it was observed that in every case iron was not employed in the preparations.[4]

TABLE 3

Humic acid substitution by ferric citrate and ferric sulfate (Erlenmeyer-Warburg technique)

	SUBSTANCE ADDED								
	None	H. A.-V		Fe-citrate			$Fe_2(SO_4)_3$		
Concentration (p.p.m.)	...	50	25	10	2	1	150	10	2
Concentration of Fe (p.p.m.)	...	0.7	0.35	2	0.4	0.2	30	2	0.4
Experiment 3:									
Final respiration rate*	173	484	......	......	528	......	......	368	......
H. A.-ratio	...	2.80	......	......	3.06	......	......	2.13	......
Experiment 3A:									
Source of N: N_2:									
Final respiration rate*	53	......	73	......	......	78	......	......	70
H. A.-ratio	...	......	1.38	......	......	1.47	......	......	1.32
Source of N: 200 p.p.m. urea-N:									
Final respiration rate*	227	......	449	......	......	537	......	......	496
H. A.-ratio†	...	......	1.98	......	......	2.36	......	......	2.18
Experiment 3B:									
Final respiration rate*	123	......	246	196	......	......	160	......	......
H. A.-ratio	...	......	2.0	1.59	......	......	1.30	......	......

Experiments 3; 3A; 3B: Initial culture 1-day; 5-hour; 1-day old *A. vinelandii*. Initial respiration rate 62; 20; about 2 cmm. O_2/cc./hr. Analyzed after 18; 48; 48 hours. Final pH 7.1 ± 0.3; N_2 6.8 ± 0.1, urea-N 6.3 ± 0.2; 6.6 ±0.1. Source of nitrogen: 500 p.p.m. urea-N; N_2 and urea; N_2. VI/3–4/31; V/11–13/31; V/23–25/31. 2 per cent glucose employed in experiment 3B (instead of 1 per cent).

* The cultures were grown in *Erlenmeyer* flasks (28°C., except Experiment 3, 31°C.) and the final relative growths measured as respiration rates (31°C.).

† It is probable that the much larger ratios obtained in urea-N (as compared to N_2) occur because of the very much greater growth and the consequently much greater need for iron.

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[4] Blumenberg and Blumenberg (4) have, however, recently patented a process for making an organic iron compound suitable as a non-toxic plant stimulant, comprising treating comminuted cellulose material (sawdust, rice-hulls, ground corn-cobs, etc.) with an aqueous solution of iron sulfate and heating to about 120°C. until a dark brown or black color is assumed. We are unaware of any experimental work performed by Blumenberg and Blumenberg in connection with this patent, but its implications obviously receive strong scientific support from our work on the preparation of active synthetic humic acids and the greater physiological availability of organic iron compounds.

As shown in table 15A, the efficacy of a natural humic acid may be increased by increasing its iron content, just as in the case of a synthetic humic acid. The final amount of growth obtained increases with the iron added, regardless of from which humic acid the iron is derived.

TABLE 4

Humic acid substitution by organic and inorganic iron compounds (Warburg technique)

	SUBSTANCE ADDED									
	None	H. A.-II					Fe-citrate		Fe-tartrate	$Fe_2(SO_4)_3$
Concentration (p.p.m.)	...	125	50	40	32	16	50	32	50	10
Concentration of Fe (p.p.m.)	...	0.9	0.35	0.3	0.2	0.1	10	6	10	2
Experiment 4:										
Respiration rate 25th hour	128	......	......	204	......	......	223	......	234	232
H. A.-ratio	...	......	......	1.59	......	......	1.74	......	1.83	1.81
Experiment 4A:										
Respiration rate 25th hour	124	....	153	.....	......	......	149	......	......	151
H. A.-ratio†	...	......	1.24	......	....	.....	1.20	.....	.....	1.22
Experiment 4B:										
Respiration rate 25th hour	147	......	323	......	......	......	342...	......	......	....
H. A.-ratio	...	......	2.20	......	......	......	2.32	......	......	....
Experiment 4C:										
Respiration rate 67th hour	137	287	......	......	212	179	......	250	......	....
H. A.-ratio	...	2.10	......	......	1.55	1.31	......	1.83	......	....

Experiments 4, 4A, 4B, 4C: Initial culture: 2-day old *A. vinelandii* diluted 3 times with customary medium containing 1 per cent glucose; 1½-day culture diluted 6 times with phosphate mixture containing 1 per cent glucose; 1½-day old culture diluted 6 times with customary medium containing 1 per cent glucose; 6-hour culture. Initial respiration rate (measured as cmm. O_2/cc./hr.): 10; 4; about 5; less than 1. Final pH 7.1 ±0.1; 7.1 ±0.1; 7.1 ±0.1; 6.8 ±0.1. Source of nitrogen: 50 p.p.m. NO_3-N; 50 p.p.m. NO_3-N; 50 p.p.m. NO_3-N; N_2. VIII/1–2/30; VIII/3–4/30; VIII/3–4/30; VIII/7–10/30.

† The H. A.-ratios are relatively low here because elements normally present in customary medium are relatively lacking.

Table 2 indicates that the growth stimulation effected by humic acid and by organic and inorganic iron compounds was not obtainable by use of certain other heavy metal compounds somewhat related to iron and sometimes suggested as being the cause of humic acid stimulation. The humic acid ratio, for the various concentrations of iron, 0.1 to 10 p.p.m., ranges from 1.34 at 0.02 p.p.m., to a maximum of 3.02 at 3 p.p.m., whereas the ratio for 0.1 to 10 p.p.m.

of the metals of the salts $CoSO_4$, $MnCl_2$, $Al_2(SO_4)_3$, $NiCl_2$, $ZnCl_2$, $Cr_2(SO_4)_3$, and Na_2MoO_4 is about 1.0 or less. Distinct toxicity was observed with 10 p.p.m. Co, 10 and 1 p.p.m. Ni, 10 p.p.m. Zn, and 10 p.p.m. Cr. In still other experiments (*Warburg* technique) of short duration (4–6 hours) no beneficial effect was given by the following metals: Mo (as Na_2MoO_4)0.5, 5, and 50 p.p.m. of metal; Cu (as $CuSO_4$) 0.01, 0.5, and 2 p.p.m.; Ag (as Ag_2SO_4) 0.05, 0.5, and 5 p.p.m.; Al (as $l_2(SO_4)_3$), 0.05, 0.5, and 5 p.p.m.; and Mn (as

TABLE 4D

Humic acid substitution by organic and inorganic iron compounds (Warburg technique)

	SUBSTANCE ADDED									
	None	H. A.-I*	H. A.-II	Fe-citrate				Fe-tartrate	$Fe_2(SO_4)_3$	
Concentration (p.p.m.)........	..	40	40	100	50	20	5	50	30	10
Concentration of Fe (p.p.m.).....	..	0.3	0.3	20	10	4	1	10	6	2
Respiration rate 25th hour (cmm. O_2/cc. hour) (relative growth)...	48	159	159	186	158	146	145	174	161	156
H. A.-ratio.......	..	3.31	3.31	3.89	3.29	3.04	3.02	3.62	3.35	3.25

Two-day old *A. vinelandii* diluted 4 times with customary medium containing 1 per cent glucose. Initial respiration rate 12 cmm. O_2/cc./hour. Final pH 7.0 ±0.1. Source of nitrogen: 50 p.p.m. NO_3-N. VII/25–26/30.

* The sample of this humic acid was 1 year old, the other sample (H. A.-II) was freshly prepared.

TABLE 4E

Humic acid substitution by organic and inorganic iron compounds (Warburg technique)

	SUBSTANCE ADDED									
	None	H. A.-II		Fe-citrate					$Fe_2(SO_4)_3$	
Concentration (p.p.m.)........	...	12	6	50	25	12	6	3	10	5
Concentration of Fe (p.p.m.).....	...	0.08	0.04	10	5.0	2.5	1.2	0.6	2.0	1.0
Respiration rate 36th hour (cmm. O_2cc./hour) (relative growth)....	129	264	181	213	214	215	230	212	199	163
H. A.-ratio.......	...	2.05	1.40	1.65	1.66	1.67	1.78	1.64	1.54	1.26

One-half day old *A. vinelandii* undiluted. Initial respiration rate about 5 cmm. O_2/cc./-hour. Final pH 7.0 ±0.1, except 10 p.p.m. $Fe_2(SO_4)_3$, where 6.0 (Initial pH all 7.0). Source of nitrogen: N_2. VIII/11–12/30.

$MnCl_2$) 0.05, 0.5, and 5 p.p.m. Under these experimental conditions definite toxicity was observed with 1 p.p.m. of Cu, and complete toxicity in one-half hour with 2 p.p.m.; Al at 5 and 0.5 p.p.m. was definitely toxic, Mo at 50 p.p.m.

TABLE 5

Humic acid substitution by various concentrations of organic iron (Erlenmeyer technique)

	SUBSTANCE ADDED								
	None	Fe-citrate					H. A.-II			
Concentration (p.p.m.)	....	0.2	1	3.2	8	25	1	2.5	8	25
Concentration of Fe (p.p.m.)	....	0.04	0.20	0.64	1.6	5.0	0.007	0.017	0.054	0.17
Final gross turbidity	21.8	28.6	36.6	42.7	48.5	50.8	31.4	56.2	86.8	100
Turbidity uninoculated controls	10.0	10.0	11.0	13.0	15.0	18.4	11.0	12.3	17.9	31.8
Final net turbidity (relative growth)	11.8	18.6	25.6	29.7	33.5	32.4	20.4	43.9	68.9	68.2
H. A.-ratio	....	1.58	2.17	2.51	2.84	2.75	1.73	5.83	3.72	5.79

Inoculated with 1 drop 1-day old *A. vinelandii*. Analyzed after 48 hours. Final pH 6.8 ±0.1. Source of nitrogen: 45 p.p.m. NO_3-N. X/11–13/30.

TABLE 6

Humic acid substitution in relatively old cultures by various concentrations of inorganic iron (Erlenmeyer technique)

	SUBSTANCE ADDED								
	None	H. A.-II		$Fe_2(SO_4)_3$					
Concentration (p.p.m.)	....	25	5	500	150	100	40	20	10
Concentration of Fe (p.p.m.)	....	0.17	0.034	100	30	20	8	4	2
Experiment 6:									
Final net turbidity*	75	176†	115		256	158	122		94
H. A.-ratio	.....	2.35	1.54		3.42	2.11	1.63		1.2
Experiment 6A:									
Final relative Kjeldahl nitrogen H. A.-ratio	1.00			1.30		2.44	3.00	3.04	2.3

Experiments 6, 6A: Inoculated with 2 drops 1-day old, 2 drops 1-day old *A. vinelandii*. Analyzed after 6 days, 6 days. Final pH 7.1 ±0.1, 6.8 ±0.1. Source of nitrogen: 200 p.p.m. NO_3-N, N_2. X/2–8/30, V/23–29/31.

* At 2 days the amount of growth in the presence of $Fe_2(SO_4)_3$ was indistinguishable from that in the control; the growth in the humic acid cultures was, of course, superior.

† It is probable, on the basis of other similar experiments, that 100 p.p.m. H. A.-II would have given a turbidity of 200–250, or a H. A.-ratio of 2.6–3.3.

very slightly toxic, and Ag and Mn were without either positive or negative effect at all concentrations studied. These *Warburg* technique experiments were conducted simultaneously with three different forms of nitrogen, urea-N (50 p.p.m.), NO_3-N (50 p.p.m.), and N_2, with identical results.

Table 8 and table 7, column 5, show that citrate, as sodium citrate, has no stimulative effect, *per se*, upon the growth of Azotobacter, under such circumstances that humic acid and ferric citrate give a large stimulation. The stimulative effect of ferric citrate is, therefore, to be referred directly to the iron fraction, the citrate fraction (or likewise tartrate, oxalate fraction, etc.) acting indirectly by holding the iron in a complex soluble form and tending to make it more soluble than inorganic iron.

TABLE 7

Humic acid substitution in bicarbonate medium by organic and inorganic iron compounds (Erlenmeyer technique)

	SUBSTANCE ADDED						
	None	H. A.-I	Fe-citrate	Na-citrate*	Fe-tartrate	Fe-oxalate	$Fe_2(SO_4)_3$
Concentration (p.p.m.)	....	25	25	25	25	25	10
Concentration of Fe (p.p.m.)	....	0.17	5	....	5	5	2
Final gross turbidity	15.6	104	75.5	13.1	67.3	72.0	37.9
	13.1	107	64.0	14.2	65.0	77.3	39.4
	11.4	98	....	15.6	....	79.5	....
Uninoculated control	10.5	8.5	9.3	11.4	6.6	13.3	32.8
Final average net turbidity 4 days (relative growth)	2.9	94	60.5	2.9	54.5	63.0	5.9
H. A.-ratio†	....	32.4	20.9	....	18.8	21.7	2.3

Inoculated with 1 drop 3 day old *A. vinelandii.* Analyzed after 4 days. Final pH 7.5 ±0.1. Source of nitrogen: 50 p.p.m. NO_3-N. VII/14-18-30.

* See also table 8.

† It is to be noted that the H. A.-ratios are exceptionally large in this experiment. These are occasionally obtained in bicarbonate medium and are due to a more pronounced lag phase in the controls. Probably by the fifth day the H. A.-ratios would have been approximately normal.

TABLE 7A

Humic acid substitution in bicarbonate medium by organic and inorganic iron compounds (Erlenmeyer technique)

	SUBSTANCE ADDED					
	None	H. A.-I	Fe citrate	Fe-tartrate	Fe-oxalate	$Fe_2(SO_4)_3$
Concentration (p.p.m.)	....	25	25	25	25	10
Concentration of Fe (p.p.m.)	....	0.17	5	5	5	2
Final gross turbidity	42.5	80.4	44.7	116	82.9	73.5
Turbidity uninoculated controls	14.7	16.7	7.8	7.5	18.4	43.5
Final net turbidity 4 days (relative growth)	27.8	63.7	36.9	109	64.5	30
H. A.-ratio	....	2.29	1.33	3.92	2.32	1.08

Inoculated with 1 drop 1-day old *A. vinelandii.* Analyzed after 4 days. Final pH 7.4 ±0.1. Source of nitrogen: 50 p.p.m. NO_3-N. VIII/2-6/30.

TABLE 7B

Humic acid substitution in bicarbonate medium by organic and inorganic iron compounds (Erlenmeyer technique)

P.p.m. of $Fe_2(SO_4)_3$	......	......	....	....	....	....	....	....	....	....	....	10	5	1
P.p.m. of H. A.-I.	100	25	12.5	2.5	0.25	....	....	....	....	....	....	....	....	....
P.p.m. of Fe-citrate	......	......	....	....	....	100	25	5	1	0.25	....	....	....	....
P.p.m. of Fe	0.68	0.17	0.08	0.02	0.002	20	5	1	0.2	0.05	....	2	1	0.2
Final gross turbidity	100	102	83.8	36.6	32.4	89.0	70.0	72.1	42.4	27.9	35.3	70.0	67.7	39.4
	101	98	93.0	35.9	29.6	83.8	80.6	79.5	.....	26.8	32.2	74.4	67.3	48.3
Turbidity uninoculated control	13.4	5.8	2.8	2.9	2.3	7.1	3.9	2.8	2.4	2.1	2.3	27.4	18.1	7.4
Final average, net turbidity (relative growth)	87.6	94.2	85.6	33.3	28.7	79.3	71.4	73.0	40.0	25.2	31.5	44.8	49.3	36.5
H. A. ratio	2.78	2.99	2.72	1.06	0.91	2.52	2.26	2.32	1.27	0.80	.....	1.42	1.57	1.16

Inoculated with 1 drop of 2-day old *A. vinelandii*. Analyzed after 48 hours. Final pH 7.5 ± 0.1. Source of nitrogen: 50 p.p.m. NO_3-N. VI/21–23/30.

Volumes of culture solution 34 cc., not 25 cc., as usual.

Sugar added after, not before, sterilization (as sterilized sugar).

In connection with tables 6A, 6, and 2, it is of interest that Elvehjem (18) has obtained similar inorganic iron stimulation with respect to baker's yeast, the humic acid ratios varying from 2.5 to 3.5. In one experiment, only 0.176 gm. dry weight per 200 cc. was obtained in a nutrient medium containing less than 0.025 p.p.m. Fe,[5] but 0.544 gm. (H. A.-ratio = 3.1) with 1 p.p.m. Fe, the duration of the experiment being 40 hours.

We have recently found (unpublished data) that under certain specific conditions (in brief, iron starvation in heavy cultures) the iron requirement of cultures grown in free nitrogen is from 2 to 10 times as great as that of cultures grown in fixed nitrogen. Under these conditions humic acid may be said to

TABLE 8

Humic acid substitution not provided by sodium citrate (Erlenmeyer techn.*

	SUBSTANCE ADDED			
	None	Na-citrate		H. A.-I
Concentration (p.p.m.)	....	25	100	25
Final relative turbidity	37.3	44.4	31.3	108
	33.9	44.8	36.2	111
	32.9	40.0	36.2	114
Uninoculated controls	4.4	8.9	8.1	11.7
	4.5	5.7	8.1	11.7
Final net turbidity (relative growth)	30.2	35.8	26.6	99.0
H. A.-ratio	....	1.18	0.88	2.26

Inoculated with 1 drop 2-day old *A. vinelandii.* Analyzed after 41 hours. Final pH 6.8 ±0.2. Source of nitrogen: N_2. V/22–24/30.

* See also table 7, column 5.

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[5] Likewise, Krzemieniewsky (29) obtained very poor growths of Azotobacter in a medium containing no humic acid, but, here again, as in the case of Beijerinck's medium employed by Remy and Rösing (39) no iron was normally intentionally added. Ruhland (40) found that for the autotrophic hydrogen bacterium, *B. pycnoticus*, 0.07 p.p.m. Fe in the culture medium was necessary for normal growth, and that at 0.0005 p.p.m. growth and hydrogen consumption no longer took place. In our judgment, based upon a consideration of the literature (see especially "Preparation of Humic Acid" paragraph 2), many higher and lower plants grown in synthetic media will respond similarly, as in the case of Azotobacter and yeast, to treatment with various of the iron compounds considered in this paper. In other words, probably few plant media are optimal with respect to the form and concentration of iron supplied, whence the occurrence of growth stimulation by inorganic, organic, or humic acid iron, as the case may be. Students of Azotobacter physiology cannot fail to have observed the wide variation in amounts of nitrogen fixed per gram of sugar per 100 cc. medium, as reported by various investigators. A considerable number report consistent values of only 1 to 3 mgm., others report 10–15 mgm. We are confident that the variations often depend in great part upon the corresponding wide differences in concentrations of iron supplied either intentionally as inorganic iron (0–100 p.p.m.) or unintentionally as impurity in the sugar added. The iron supplied in the latter manner is an important factor in Azotobacter growth and is not so completely precipitated as the same concentration of purely inorganic iron, i.e., iron in sugar is partly in organic combination.

TABLE 9

The substitution of natural by synthetic humic acids (Erlenmeyer technique)

	SUBSTANCE ADDED																	
	None	H. A.-VII				H. A.-V				H. A -IV			H. A.-III			H. A.-VI		
Description...........		Synthetic medium-Fe				Natural high-Fe				Natural medium-Fe			Natural medium-Fe			Synthetic low-Fe		
Concentration (p.p.m.).........	..	100	25	10	5	100	25	10	1	100	25	10	100	25	10	100	25	10
Concentration of Fe (p.p.m.)......	..	0.30	0.075	0.03	0.015	1.4	0.35	0.15	0.014	0.11	0.03	0.11	0.06	0.15	0.006	0.03	0.008	0.003
Final net turbidity (relative growth).	41	285	163	121	115	260	137	105	67	103	97	61	71	83	51	84	80	60
H. A.-ratio:																		
100 p.p.m.......	..	6.95	......	......	......	6.34	......	......	......	2.51	......	......	1.73	......	......	2.05	......	......
25 p.p.m........	..	......	3.98	......	......	......	3.34	......	......	......	2.36	......	......	2.02	......	......	1.95	......
10 p.p.m........	..	......	......	2.95	......	......	......	2.56	......	......	......	1.49	......	......	1.24	......	......	1.46
1 or 5 p.p.m.....	..	......	......	......	2.80	......	......	......	1.63	......	......	......	......	......	......	......	......	......

Inoculated with 1 drop 2-day old *A. vinelandii*. Analyzed after 2 days. Final pH 6.6 ± 0.2. Source of nitrogen: N_2. XI/5–7/30.

TABLE 9A-B

The substitution of natural by synthetic humic acids containing iron (Erlenmeyer Technique)

	SUBSTANCE ADDED												
	None	H. A.-V			H. A.-VII			H. A.-IV			H. A.-VI		
Description		Natural high-Fe			Synthetic medium-Fe			Natural medium-Fe			Synthetic low-Fe		
Concentration (p.p.m.)	.....	250	50	18	250	50	18	250	50	18	250	50	18
Concentration of Fe (p.p.m.)	.....	3.5	0.70	0.25	0.75	0.15	0.05	0.27	0.05	0.02	0.10	0.02	0.005
Experiment 9B:													
Final net turbidity	25.3	......	......	46.8	......	......	48.8	......	......	40.0	......	......	22.0
H. A.-ratio	.....	......	......	1.85	......	......	1.93	......	......	1.58	......	......	0.87
Experiment 9A:													
Final net turbidity	285	658	557	.....	691	537	.....	581	475	.....	405	391	......
H. A.-ratio	.....	......	......	.....	......	......	.....	......	......	.....	......	......	......
50 p.p.m.	.....	......	1.95	.....	......	1.89	.....	......	1.67	.....	......	1.37	......
250 p.p.m.	.....	2.31	......	.....	2.42	......	.....	2.04	......	.....	1.42	......	......

Experiments 9A, 9B: Inoculated with 1 drop 2-day old; 1 drop 2-day old *A. vinelandii.*
Analyzed after 11 days; 48 hours. Final pH 6.5 ± 0.1; 6.4 ± 0.1. Source of nitrogen: N_2; 200 p.p.m. NO_3-N. 1/17–28/31; XII/10–12/30.

TABLE 10

The substitution of natural by synthetic humic acids (Warburg technique)

	SUBSTANCE ADDED		
	None	H. A.-V	H. A.-VII
Description		Natural high Fe	Synthetic medium-Fe
Concentration (p.p.m.)	...	25	50
Concentration of Fe (p.p.m.)	...	0.35	0.15
Experiment 1:*			
Respiration rate 14th hour (cmm. O_2/cc./hour) (relative growth)	80	100	105
H. A.-ratio	...	1.25	1.31
Experiment 2:†			
Respiration rate 34th hour	196	260	260
H. A.-ratio	...	1.33	1.33
Experiment 3‡:			
Respiration rate 34th hour	77	255	246
H. A.-ratio	...	3.31§	3.19

* Thirty-six-hour old *A. vinelandii* diluted 5 times with customary medium containing 1 per cent glucose. Initial respiration rate 10 cmm. O_2/cc./hour. Final pH 6.7 ±0.1. Source of nitrogen: N_2. I/12/31.

† Six-hour old *A. vinelandii*. Initial respiration rate about 2 cmm. O_2/cc./hour. Final pH 6.8 ±0.1. Source of nitrogen: N_2. I/13–14/31.

‡ Thirty-hour old *A. vinelandii* diluted 40 times with customary medium containing 1 per cent glucose. Initial respiration rate less than 1 cmm; O_2/cc./hour. Final pH 6.8 ±0.1. Source of nitrogen: N_2. I/13–14/31.

§ Fe-citrate (25 p.p.m. = 5 p.p.m. Fe) gave simultaneously a humic acid ratio of 3.41.

TABLE 10A

The substitution of natural by synthetic humic acids (Warburg technique)

	SUBSTANCE ADDED				
	None	H. A.-V	H. A.-VII	H. A.-VI	Fe-citrate
Description		Natural high Fe	Synthetic medium Fe	Synthetic low-Fe	
Concentration (p.p.m.)	....	25	50	50	5
Concentration of Fe (p.p.m.)	....	0.35	0.15	0.02	1.0
Final net turbidity 22nd hour (relative growth)	11.8	60.1	31.4	14.2	52.7
H. A.-ratio	....	5.10	2.66	1.20	4.47

Two-day old *A. vinelandii*. Final pH 6.2 ±0.2. Source of nitrogen: N_2. I/15/31. The cultures were grown in Warburg vessels and analyzed nephelometrically.

possess a specific effect on the chemical mechanism of nitrogen fixation, insofar as it provides iron. This iron may, however, be provided (as we have found) qualitatively as well by synthetic iron-containing humic acids, iron-organic

TABLE 11

The substitution of natural by synthetic humic acids (Erlenmeyer-Warburg technique)

	SUBSTANCE ADDED				
	None	H. A.-V	H. A.-IX	H. A.-X	Fe-citrate
Description		Natural high-Fe	Synthetic high-Fe	Synthetic high-Fe	
Concentration (p.p.m.)	....	10	10	10	10
Concentration of Fe (p.p.m.)	....	0.14	0.42	0.32	2
Final respiration rate* (cmm. O_2/cc./hour) (relative growth)	114	175	261	252	224
H. A.-ratio	....	1.54	2.29	2.22	1.97

Initial culture 2-day old *A. vinelandii* to which was added 1 per cent glucose and 0.1 per cent phosphate mixture. Initial respiration 94 cmm. O_2/cc./hour. Analyzed after 6 days. Final pH 6.7 ±0.1. Source of nitrogen: N_2. V/17–23/31.

* The cultures were grown in *Erlenmeyer* flasks (28°C). and the final relative growths measured as respiration rates (31°C.).

TABLE 11A

The substitution of natural by synthetic humic acids (Erlenmeyer-Warburg technique)

	SUBSTANCE ADDED								
	None	H. A.-V		H. A.-VIII		H. A.-X		Fe-citrate	$Fe_2(SO_4)_3$
Description		Natural high-Fe		Synthetic high-Fe		Synthetic low-Fe			
Concentration (p.p.m.)	...	5	25	5	25	5	25	1	2
Concentration of Fe (p.p.m.)	...	0.07	0.35	0.002	0.01	0.16	0.80	0.2	0.4
Experiment 1 (N_2):									
Respiration rate 18th hour (cmm. O_2/cc./hour) (relative growth)	61	103	122	67	90	93	103	65	65
H. A.-ratio	...	1.69	2.00	1.10	1.48	1.53	1.69	1.06	1.06
Experiment 2 (200 p.p.m. urea-N):									
Respiration rate 18th hour	163	186	187	160	168	200	220	209	232
H. A.-ratio	...	1.14	1.15	0.98	1.03	1.23	1.35	1.28	1.42
Experiment 3 (200 p.p.m. urea-N):									
Respiration rate 46th hour	236	......	449	301	300	506	600	537	496
H. A.-ratio	...	......	1.90	1.28	1.27	2.14	2.54	2.28	2.10
pH ±0.2 (experiment 3)	7.4	......	6.8	7.4	7.4	6.8	6.2	6.2	6.1

Initial culture 5-hour old *A. vinelandii.* Initial respiration rate 20 cmm. O_2/cc./hour. Final pH 6.7 ±0.2, except where indicated in experiment 3. V/11–13/31.

TABLE 12

Comparison of the effects of different concentrations of iron-rich and iron-poor humic acids on growth (Erlenmeyer technique)

	SUBSTANCE ADDED														
	None	H. A.-V		H. A.-IX		H. A.-X		H. A.-XI		H. A.-VII		H. A.-VI		H. A.-VIII	
Description		Natural high-Fe		Synthetic high-Fe		Synthetic high-Fe		Synthetic high-Fe		Synthetic medium-Fe		Synthetic low-Fe		Synthetic low-Fe	
Concentration (p.p.m.)	...	10	60	10	60	10	60	10	60	10	60	10	60	10	60
Concentration of Fe (p.p.m.)	...	0.14	0.84	0.42	2.52	0.32	1.92	0.44	2.64	0.03	0.18	0.003	0.018	0.003	0.018
Final net turbidity (relative growth)	270	325	370	318	534	330	591	379	600	300	372	240	327	265	356
H. A.-ratio (10 p.p.m.)	...	1.20	....	1.18	.....	1.22	......	1.40	......	1.11	......	0.89	....	0.98	....
H. A.-ratio (60 p.p.m.)	...	......	1.37	.....	1.98	......	2.19	......	2.22	......	1.38	......	1.21	......	1.32

Inoculated with 1 drop 1-day old *A. vinelandii*. Analyzed after 6 days. Final pH 6.7 ±0.1 Source of nitrogen: N_2. I/28–II/4/31.

TABLE 13

Comparison of effects of iron-rich and iron-poor humic acids on growth (Warburg technique)

	SUBSTANCE ADDED					
	None	H. A.-V			H. A.-X	H. A.-XIX
Description		Natural high-Fe			Synthetic high-Fe	Synthetic low-Fe
Experiment 1:*						
Concentration (p.p.m.)	..	225	75	25	225	225
Concentration of Fe (p.p.m.)	..	3.15	1.05	0.35	7.2	0.07
Respiration rate 25th hour (cmm. O_2/cc./hour) (relative growth)	95	350	276	199	200	116
H. A.-ratio	..	3.68	2.91	2.10	2.10	1.22

	SUBSTANCE ADDED						
	None	H. A.-V		H. A.-X		H. A.-XIX	
Description		Natural high-Fe		Synthetic high-Fe		Synthetic low-Fe	
Experiment 2:†							
Concentration (p.p.m.)	...	50	2	50	2	50	2
Concentration of Fe (p.p.m.)	...	0.70	0.03	1.6	0.06	0.015	0.0006
Respiration rate 12th hour	160	340	168	243	173	187	173
H. A.-ratio 50 p.p.m.	...	2.12	.....	1.52	......	1.17	....
H. A.-ratio 2 p.p.m.	...	......	1.05	......	1.08	....	1.08

	SUBSTANCE ADDED					
	None	H. A.-V		H. A.-X	H. A.-VIII	Fe-citrate
Description		Natural high-Fe		Synthetic high-Fe	Synthetic low-Fe	
Experiment 3:‡						
Concentration (p.p.m.)	...	50	10	10	10	10
Concentration of Fe (p.p.m.)	...	0.70	0.14	0.32	0.003	2
Respiration rate, 13th hour	137	236	205	203	159	225
H. A.-ratio	...	1.72	1.50	1.48	1.16	1.64

* Initial culture 2-day old *A. vinelandii.* Initial respiration rate 60 cmm. O_2/cc./hour. Final pH 7.0 ±0.1. Source of nitrogen: N_2. VII/13–14/31.

† Initial culture 1-day old *A. vinelandii.* Initial respiration rate 60 cmm. O_2/cc./hour. Final pH 6.6 ±0.1. Source of nitrogen: N_2. VII/9/31.

‡ Initial culture 1-day old *A. vinelandii.* Initial respiration rate 30 cmm. O_2/cc./hour. Final pH 7.0 ±0.2. Source of nitrogen: N_2. V/8/31.

acids, etc., but is not supplied by iron-free humic acids. So far as concerns this paper, the findings provides strong additional proof that natural humic acid acts by virtue of its iron component, since humic acid definitely alleviates a specific case of proved iron-deficiency.

TABLE 14

Comparison of iron-humic acids with other metal-humic acids (Erlenmeyer technique)

		20 P P M OF H. A.												
		V	VI	VII	IX	X	XI	XII	XIV	XV	XVI	XVII	XVIII	XIX
Description..		Natural high-Fe	Synthetic low-Fe	Synthetic medium-Fe	Synthetic high-Fe	Synthetic high-Fe	Synthetic high-Fe	Synthetic low-Fe	Synthetic low-Fe	Synthetic low-Fe	Synthetic low-Fe	Synthetic low-Fe	Synthetic low-Fe	Synthetic low-Fe
Metal added in preparation of H. A.				Fe	Fe	Fe	Fe	Mo	P	Si	Co	Mn		
P.p.m. of Fe	...	0.28	0.006	0.06	0.84	0.64	0.87	0.006	0.006	0.006	0.006	0.006	0.006	0.006
Turbidity 42 hours (relative growth)	39.3*	100	44.6	67.6	75.7	80.6	161	46.9	52.9	58.0	53.2	56.5	46.1	42.0
	100													
Turbidity 66 hours	90.8	308	103	172	215	208	240	107	124	148	125	136	117	114
	87.2	292	109	174	215	240	224	109	136	162	140	130	111	110
Average	92.4	300	105	173	215	224	232	108	129	154	132	133	114	112
H.A.-ratio 42 hours	—	2.55	1.14	1.72	1.93	2.05	4.10	1.19	1.35	1.48	1.35	1.44	1.17	1.07
H.A.-ratio 66 hours	—	3.25	1.14	1.87	2.32	2.42	2.51	1.17	1.40	1.67	1.43	1.43	1.23	1.21

Inoculated with 4 drops 2-day-old *A. vinelandii*. Final pH 6.6 ±0.2. Source of nitrogen: N_2. VII/15–21/31.

* Average of three determinations 41.0, 38.9, and 37.9.

H. A.-XIII containing Al caused inhibition of growth.

TABLE 14A-B

Comparison of iron-humic acids with other metal-humic acids (Erlenmeyer-Warburg technique)

		HUMIC ACID										
		V	IX	X	XVI	XIV	XV	XVII	XII	XVIII	VI	Fe-citrate (10 p.p.m.)
Description of humic acid		Natural high-Fe	Synthetic high-Fe	Synthetic high-Fe	Synthetic low-Fe	Synthetic low-Fe	Synthetic low-Fe	Synthetic low-Fe	Synthetic low-Fe	Synthetic low Fe	Synthetic low Fe	
Metal added in preparation of H.A			Fe	Fe	Co	P	Si	Mn	Mo			
Experiment 14A (25 p.p.m. H. A.):												
P.p.m. Fe	...	0.35	......	0.80	0.008	0.008	0.008	0.008	0.008	0.008	0.008	......
Final respiration rate*	123	246	......	212	187	178	175	169	166	162	140	......
H. A.-ratio	...	2.00	.....	1.72	1.52	1.45	1.42	1.37	1.35	1.32	1.14	......
Per cent increase relative to H. A.-XVIII	...	51.8	......	30.8	15.4	9.9	8.0	4.3	2.5	......	......	......
Experiment 14B (10 p.p.m. H. A):												
P.p.m. Fe	...	0.14	0.42	0.32	0.003	0.003	0.003	0.003	0.003	0.003	......	2.0
Final respiration rate*	114	175	261	252	134	172	144	161	155	129	......	224
H. A.-ratio	...	1.54	2.29	2.22	1.18	1.51	1.26	1.41	1.36	1.13	......	1.97

Experiments 14A, 14B: Initial culture 1 cc. 1-day old *A. vinelandii* diluted 5 times with sterile customary medium; 2-day old *A. vinelandii* to which was added 1 per cent glucose and 0.1 per cent phosphate mixture. Initial respiration rate about 2 cmm. O_2/cc./hour, 94 cmm./cc./hour. Analyzed after 2, 6 days. Final pH 6.6 ±0.1; 6.7 ±0.1. Source of nitrogen: N_2; N_2. V/23–25/31; V/17–23/31. 2 per cent glucose in experiment 14A instead of 1 per cent.

* The cultures were grown in *Erlenmeyer* flasks (28°C.) and the final relative growths measured as respiration rates (31°C.) as cmm. O_2/cc./hour. H. A.-XIII, containing Al, caused inhibition of growth.

TABLE 15

The influence of duration of experiment upon the humic acid ratio with natural and synthetic humic acids (Erlenmeyer technique)

	SUBSTANCE ADDED (25 P.P.M.)								
	None			H. A.-V			H. A.-VII		
Description				Natural high Fe			Synthetic medium-Fe		
Concentration of Fe (p.p.m.)	....	....	...	0.35	0.35	0.35	0.075	0.075	0.075
Duration (days)	2	3	5	2	3	5	2	3	5
Final net turbidity (relative growth)	10.0	68.1	160	18.6	100	219	23.4	117	243
H. A.-ratio	....	....	...	1.86	1.47	1.37	2.34	1.72	1.52

Inoculated with 1 drop 2-day old *A. vinelandii.* Analyzed after 2, 3, and 5 days. Final pH 6.3 ±0.3. Source of nitrogen: 200 p.p.m. NO_3-N. XII/10-15/30.

TABLE 15A

The improvement of natural humic acids by the addition of iron (Warburg technique)

	SUBSTANCE ADDED						
	None	H. A.-XX*		H. A.-XXI*		H. A.-XXII*	
Concentration (p.p.m.)	....	15	3	15	3	15	3
Concentration of Fe (p.p.m.)	....	0.06	0.01	0.15	0.03	0.15	0.03
Final relative turbidity 25th hour (H. A.-ratio)	1.00	1.65	1.01	2.10	1.26	2.06	1.13

Initial culture 1-day old *A. vinelandii.* Final pH, 6.8 ±0.1. Source of nitrogen: N_2. XI/6/31.

* H. A.-XX, a natural humic acid, was prepared from a garden soil (X/–/31), and contained a medium amount of iron, 0.4 per cent. H. A.-XXI and -XXII were prepared from H.A.-XX by allowing a 3.8 per cent solution of the latter containing 0.5 per cent $Fe_2(SO_4)_3\cdot 9H_2O$ in respectively (*a*) alkali (pH 9–10) and (*b*) acid (pH 3–4) to stand on the steam bath for 5 hours, at room temperature for 17 hours, and then repurifying eight times until the wash waters gave but traces of Fe H.A.-XXI and -XXII contained 1.1 ±0.1 per cent Fe, or an increase of 0.7 per cent over H.A.-XX.

THE HUMIC ACID RATIO AS A QUANTITATIVE FUNCTION OF THE IRON CONCENTRATIONS OF THE STIMULANT ADDED

Tables 16, 17, 18, and figures 2 and 3 indicate how far the qualitative humic acid substitution effects obtainable with the different iron compound types in tables 2–15 are capable of receiving quantitative functional expression and comparison. In making the comparisons, it is not the concentration of the compound, but the concentration of iron added by the compound which will be of interest. There are four different iron compound types: natural humic acid iron, synthetic humic acid iron, organic acid iron, and inorganic iron. Five compounds of the first type, 14 of the second, 3 of the third, and 2 of the fourth have been studied.

When all the experiments are considered as a whole, it is probable that, with the possible exception of inorganic iron, the other three types are approximately equivalent, within 50 per cent, in ordinary experiments lasting from one to several days, and when medium or small concentrations of stimulator are used. It is not to have been anticipated in tables 2–15 that the different iron com-

TABLE 16

The humic acid ratio as a function of concentration of iron in compounds of the organic acid iron type

DATA FROM TABLE NUMBER	Fe	HUMIC ACID RATIO		
		Fe-citrate	Fe-tartrate	Fe-oxalate
	p p m			
2	2	1.60	1.65	. ..
4	10	1.74	1.83	. .
4D	10	3.29	3.62	. .
7*	(5)	(20.9)	(18.8)	(21.7)
7A	5	1.33	3.92	2.32

* See footnote table 7.

TABLE 17

The humic acid ratio as a function of concentration of iron in synthetic humic acids

SYNTHETIC HUMIC ACID	NUMBER OF EXPERIMENTS	AVERAGE Fe CONCENTRATION	AVERAGE H. A.-RATIO
		(*p p m*)	
XVIII (nothing)	4	0.006	1.21
XII (Mo)	4	0.006	1.27
XV (Si)	4	0.006	1.36
XVII (Mn)	4	0.006	1.41
XIV (P)	4	0.006	1.42
XVI (Co)	4	0.006	1.46
XIX (nothing)	4	0.007* (0.018)	1.11* (1.15)
VIII (nothing)	4	0.007	1.18
VI (nothing)	12	0.019	1.31
VII (Fe)	18	0.141	2.39
X (Fe)	14	0.40* (1.00)	1.60* (1.84)
IX (Fe)	5	0.59* (0.91)	2.00* (2.00)
XI (Fe)	3	0.72* (1.21)	2.67* (2.56)

* These average values do not contain a few certain exceptionally high-iron value experiments (0.07 per cent Fe with H. A.-XIX; 2.64 per cent with H. A.-XI; 2.52 per cent with H. A. IX; 1.92, 1.6 and 7.2 per cent with H. A.-X) which would give misleading averages. The values in parentheses give the averages when all experiments are included. That the omitted iron values are exceptional and excessive can be seen from the fact that although the average iron concentration values are lowered considerably the average acid ratios are little affected. The same phenomenon will be observed in table 18 (except possibly with respect to inorganic iron, which discrepancy can be explained upon the basis of solubility relations).

TABLE 18

The humic acid ratio as a function of concentration of iron in the four iron-compound types (based on tables 2–15 inclusive)

	TYPE 1	TYPE 2	TYPE 3	TYPE 4
Type of iron compound	Natural H. A.	Synthetic H. A.	Organic acid	Inorganic
Compounds included	I, II, III IV, V	VII, IX, X, XI	Fe citrate, oxalate, tartrate	$Fe_2(SO_4)_3$
Average using all experiments:*				
Average concentration of Fe	0.381	0.645	4.24	8.55
Average H. A. ratio	2.20	2.15	2.19	1.97
Number of experiments	70	45	39	30
Selected average:†				
Average concentration of Fe	0.268	0.309	0.271	0.386
Average H. A.-ratio	2.10	2.09	2.14	1.57
Number of experiments	66	40	11	7

* Except that in table 7 (see footnote to table 7).

† Based upon all iron concentrations of 1 p.p.m. Fe or less (see footnote to table 17).

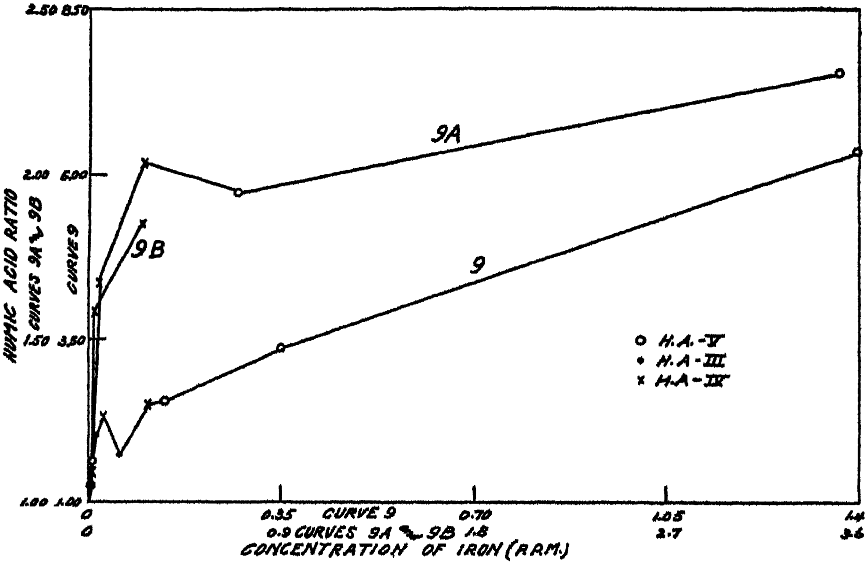

FIG. 2. THE HUMIC ACID RATIO AS A FUNCTION OF CONCENTRATION OF IRON SUPPLIED BY NATURAL HUMIC ACIDS

pounds should have all given exactly the same humic acid ratio as the control natural humic acid, either in any individual experiment, or as between different experiments. For one consideration, different natural humic acids give varying values among themselves, depending chiefly upon iron content, as shown in table 9 and figure 2. Humic acid ratios qualitatively representing stimulation of the same order of magnitude are all that can be expected in any ordinary experiment, although it is undoubtedly possible to arrange experiments to yield substantially identical ratios by paying sufficient attention to certain secondary factors. Chief of these secondary factors determining the precise quantitative relations governing the value of the ratios are, as will be for the most part evident from inspection of the tables, (*a*) the duration of the experiment [see (11), "The Integral Functions"], (*b*) the concentration of the stimulant added (see tables 2, 4C, 4D, 4E, 5, 6, 6A, 7B, 9, 9A, 11A, 12, 13, and 15), (*c*) the chemical nature of the stimulant with respect to iron content, solubility, and physiological availability, and (*d*) the time value of the induction phase.

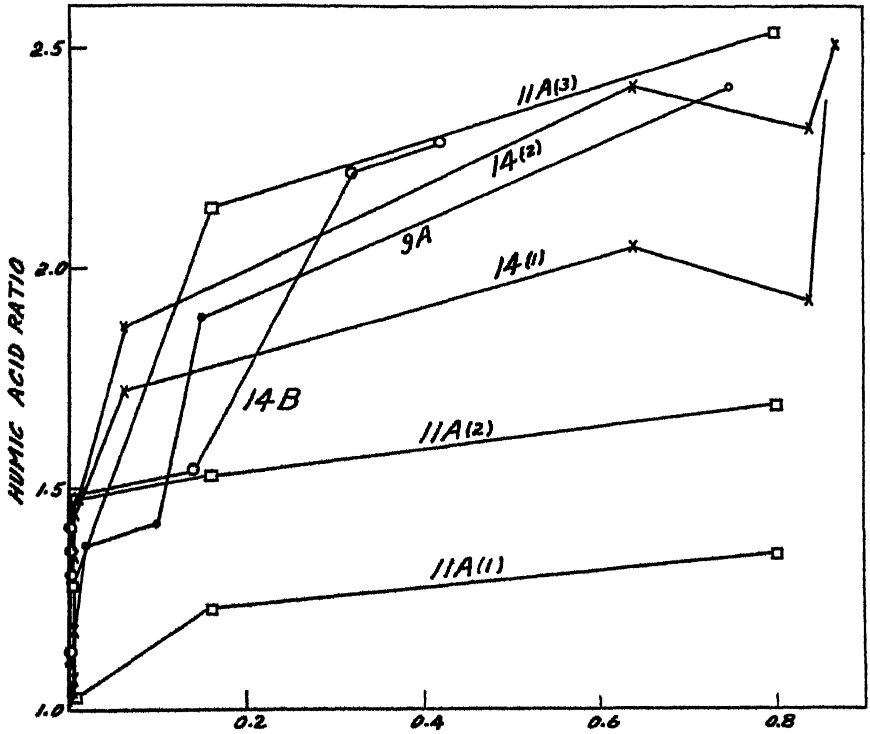

FIG. 3. THE HUMIC ACID RATIO AS A FUNCTION OF CONCENTRATION OF IRON SUPPLIED BY SYNTHETIC HUMIC ACIDS

Table 16, which contains all the data available for direct comparison, shows that, at any given concentration of iron, ferric citrate, tartrate, and oxalate give the same humic acid ratio, within reasonable limits. In most of the experiments in table 16, the iron concentration is somewhat in excess of the minimum amount required to give approximately the optimum effect.

A comparison of figures 2 and 3 shows that about 0.3–0.8 p.p.m. Fe yields about 60–80 per cent of the maximum humic acid ratio with both natural and synthetic humic acids. Curves 9, 9A, 9B, 11A, 14, 14B refer to tables 9, 9A, 9B, 11A, 14, 14B, respectively. To avoid confusion, in figure 3 we have not included all the available comparable data, but inspection of the excluded experiments (tables 9, 9B, 10A, 11, 12, 13, 14A) shows that they conform to those represented in the figure in indicating that, in general, in any given experiment, the humic acid ratio increases with the concentration of the iron added, independently of the particular synthetic humic acid employed. H. A.-VII is possibly a trifle more efficient per unit weight of iron than the other synthetic humic acids.

In table 17 are averaged the iron concentrations and respective humic acid ratios obtained in all experiments. An excellent general correlation is evident. H. A.-VII again appears to be somewhat the most efficient; but in comparing it to H. A.-IX and X, and possibly XI, one should remember that in the latter three cases considerably more iron has been employed (0.40, 0.59, and 0.72, instead of 0.14 per cent), probably more than the minimum necessary to give approximately the optimum effect. The superiority of H. A.-VII is, therefore somewhat more apparent than real. The *low*-iron synthetic humic acids containing Mo, Si, Mn, P, and Co appear to be very slightly better than those containing none of these elements (an average H. A.-ratio of 1.38 compared to 1.20), since they are all superior to about the same slight extent it is probable that these elements are not specific and that if the humic acids had been similarly prepared in the presence of more common salts, such as NaCl, $MgSO_4$, $CaSO_4$, the same slight superiority would have been obtained. At the same time we do not wish to preclude the possibility that stimulating, iron-free humic acids, containing the relatively rarer elements (Mn, Al, Co, Si, etc.) could not be prepared by some modified method. It is conceivable, for instance, that some organism being studied might have a special need for, say, aluminum, in which case a synthetic or natural humic acid containing aluminum impurity might very well act by virtue of supplying aluminum, instead of, or in addition to, iron.

Table 18 gives a comparison of the four types of iron compounds upon the basis of (*a*) all experiments, and (*b*) experiments with reasonable iron concentrations (optimal or sub-optimal, but not supra-optimal). The "Average Using All Experiments," is included as a matter of passing interest; it is quite obvious that here the H. A.-ratios of types 3 and 4 are incomparable to those of types 1 and 2 because the average iron concentrations involved are so much greater. The "Selected Average" excludes all iron concentrations above 1 p.p.m. Fe, amounting to 4 experiments (out of 70) in type 1; 5 experiments (out of 45) in type 2; 28 experiments (out of 39) in type 3, and 23 experiments (out of 30) in type 4 (see table 18, rows 6 and 9). The "Selected Average" iron concentrations are now of the same order of magnitude (0.266 to 0.386 p.p.m.) and the humic acid ratios also approximately so. The inorganic iron appears to be slightly less effective than the other three types, but it must be remembered that 0.386 p.p.m. inorganic iron would be on the average only 25–50 per cent soluble, and, when this allowance is made, the ratio of 1.57 as compared to 2.10, 2.09, and 2.14, is quite within expectation based upon concentration functions similar to those represented in figures 2 and 3.

The general conclusion that a given *sub-maximal* amount of *soluble* iron, viz., about 0.2–1.0 p.p.m. or less, gives, independently of iron-compound type, the same order of magnitude of humic acid ratio, is subject to the provision that the experiments be allowed to proceed long enough to avoid any limitation through induction, viz., 36–48 hours or more, and to consume the physiologically active iron added. Consideration of the induction period is essential at this point in order to prevent any misinterpretation of the tables regarding the exact equivalence of natural humic acids and iron compounds, especially inorganic iron. In this connection, tables 3B, 7, 7A, 7B, and 11A (exp. 1), give some indication that $Fe_2(SO_4)_3$, especially, is not exactly equivalent to ferric citrate[6] or humic acid with respect to certain time relations. The lengths of

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[6] Olsen (38) has recently indicated in a preliminary publication that humic acid may be quantitatively replaced by ferric citrate in stimulating plant growth, the experiments having been conducted with *Lemna polyrhiza* and *Helianthus annuus*. His humic acid consisted of equal parts of aqueous alkaline extract of bacterized peat and ammoniacal extracts of peat. With the former plant 183 mgm. of dry matter were obtained without humic acid, and 452 mgm. with 15 p.p.m. of humic acid (10 p.p.m. $FeCl_3$ being present in both cases, in an inorganic medium made up with distilled water); whereas, when the $FeCl_3$ was replaced with one-half the equivalent quantity of Fe-citrate (but without humic acid being present, 476 mgm. of dry matter were likewise obtained. The same results with respect to equivalence of Fe-citrate and humic acid were obtained when tap water was used instead of distilled water; moreover, Fe-citrate and humic acid together gave no higher yields than either alone, within 10 per cent; the pH in the aforementioned experiments was maintained at approximately 7.5, at which pH iron is more or less completely precipitated in the absence of organic matter. Similar results were obtained with Helianthus grown at pH 5.2 to 6.8. Saeger (42) likewise obtained stimulation effects with Spirodela and Lemna, using organic extracts derived from either a 1 per cent $NaHCO_3$ extract of peat, or a watery extract of autolyzed yeast.

In view of the apparently clear-cut nature of Olsen's experiments, it is pertinent to note that several important criticisms obtain. In the most decisive experiment, with Lemna, there were only eight experimental cultures reported, in six of which the amounts of dry matter obtained were identical within experimental error; in other words, the chief conclusion of the paper is based upon the behavior of virtually two divergent cultures. A more specific criticism, however, is that no experiments were performed in which the humic acid was added in the absence of added Fe, so that there remains the uncertainty as to (*a*) whether the growth would have been as great in the presence of humic acid and the absence of $FeCl_3$, as in the presence of both; or (*b*) conversely, whether the humic acid actually increased the availability of the $FeCl_3$ added. Also, there were no experiments where citrate was added in the absence of Fe; i.e., the effect of the Fe-citrate may have been due to the citrate entirely, so far as the experiments were capable of indicating. Finally, the experiments were not conducted under sterile conditions with respect to organisms other than Lemna; according to Clark (13, 15), under sterile conditions, the growth of Lemna is not stimulated by organic extracts.

Probably the most extensive work connecting the humic acid effect with addition of contained iron, previous to that being reported, is that of Remy and Rösing (39). These authors showed that (*a*), as the humic acid was freed from iron by chemical purification, its activity rapidly decreased to zero in solutions to which no inorganic iron had been added purposely;

the induction periods, vary from a few hours to a day or more (11, "Induction in Humic Acid Substitution Compounds"). It is obvious, therefore that in the early stages of growth, especially during the first day, those substances with the shortest induction period will for a time appear superior, often considerably so.

IS HUMIC ACID STIMULATION DUE SOLELY TO IRON?

It is true that not only do humic acid and iron compounds cause similar growth stimulations, but also, *where humic acid has no stimulative effect, iron compounds likewise have no stimulative effect.* This is shown in tables 19, 19A, 19B, and 20, 20A for Azotobacter and Chlorella, respectively, and is true even though at the same time changing other chemical substances and physiological conditions actually can produce stimulation, e.g., Azotobacter: increasing the temperature to 33-35°C., lowering the oxygen concentration to 0.05 atm., adding urea-nitrogen; Chlorella: increasing the illumination, by growing the cultures under non-stagnant conditions (i.e., with agitation by rapid aeration), by employing much more dilute inoculations, and to a limited extent, by changing the concentrations of some of the chemical constituents of the medium.

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this was shown also by Krzemieniewski (29) and Mockeridge (34); and (*b*) upon the addition of inorganic iron to a medium containing inactive humic acid the growth of Azotobacter became as great as in the presence of active humic acid. Thus it is seen that they claimed that humic acid not only added contained iron, but could, under certain circumstances, increase the availability of inorganic iron added to the medium. Unfortunately several important control experiments were neglected. With respect to (*b*) it was not shown in the same series of experiments (No. 152–159) that the inorganic iron (Fe_2O_3) added would not have given as large an effect even in the absence of inactive humic acid, yet other experiments (viz., No. 121, compared to Nos. 109 and 110) indicated that this would have been substantially the case. It was not shown that iron plus active humic acid gave results equal to, but not greater than, iron alone, to prove that the action of the active humic acid was confined to that of supplying iron. With respect to (*a*), they were unable to answer satisfactorily the objection that at the same time that the humic acid was being freed from iron it was (as they showed) likewise being freed from aluminum, phosphorus, and silica, and to about the same proportional extent, so their results really indicated only that humic acid lost its effect as something was removed or destroyed by the process of purification. The criticisms of the work of Remy and Rösing, and likewise that of Olsen's work, are advanced not with the purpose of throwing doubt upon the chief final conclusions arrived at, but with the purpose of indicating the insecurity, or rather, incompleteness of the data used in reaching the conclusions. One general criticism, perhaps the most important of any, must never be lost slight of in connection with all the work on humic acid, whether in connection with iron or other substances, by workers prior to about 1920, namely, that no pains were taken either to regulate or to observe pH changes. With Azotobacter, growth of which ceases at pH 6, and greatly decreases above pH 8, this is essential. The media employed by the earlier workers generally contained but little phosphate buffer (0.2 to 0.5 gm. potassium phosphate per liter), and with the high amounts of iron generally employed (even 100 to p.p.m.) it was inevitable that pH changes over wide ranges should occur. In attempting to interpret the earlier work consideration of the effect of pH cannot be too strongly emphasized. The report given in this footnote of the work of Olsen and of Remy and Rösing, together with our work reported in this paper, will give the reader a summarizing account of the chief *experimental* work on the relation between iron and the physiologically beneficial action of humic acid.

TABLE 19

*The absence of stimulation upon addition of humic acid or iron compounds to a very young culture of Azotobacter**

SUBSTANCE ADDED	CONCENTRATION	CONCENTRATION Fe	EXPERIMENT 19		EXPERIMENT 19A		EXPERIMENT 19B	
			Final net turbidity 24th hr.	H. A.-ratio	Final respiration rate 10th hour	H. A.-ratio	Final respiration rate 20th hour†	H. A.-ratio
	p.p.m.	*p.p.m.*						
None	......	....	41.4	....	98	....	6.8	.
H. A.-I	40	0.27	....	....	91	0.93	...	.
	25	0.17	40.7	0.99	..	....	...	.
H. A.-II	250	1.7	....	....	..	....	5.8	1
	125	0.85	....	....	..	....	5.9	1
	62	0.43	....	....	..	....	5.4	1
	31	0.21	....	....	..	....	5.3	1
	40	0.27	....	....	98	1.00	...	.
$Fe_2(SO_4)_3$	30	6	....	....	95	0.97	...	.
	10	2	....	....	93	0.95	...	.
Fe-tartrate	50	10			92	0.94		
Fe-citrate	100	20	....	....	96	0.98	...	.
	50	10	....	....	95	0.97	...	.
	20	4	....	....	90	0.92	...	.
	5	1	....	....	98	1.00	...	.
	31	6	....	....	..	....	6.0	1
	25	5	39.4	0.95	..	....	...	.
	5	1	46.4	1.12	..	....	...	.
	1	0.2	47.7	1.15	..	....	...	.
	0.25	0.05	46.2	1.12	..	....	...	.
Fe-citrate plus 25 p.p.m. H. A.-I (0.17 p.p.m. Fe)	100	20	42.6	1.03	..	....	...	.
	5	1.2	45.7	1.11	..	....	...	.
	1	0.37	41.1	1.00	..	....	...	.
	0.25	0.22	43.1	1.04	..	....	...	.

Experiments 19; 19A; 19B: Technique *Erlenmeyer; Warburg; Warburg.* Initial culture 1 drop 1-day; 1-day; 6 hour old *A. vinelandii.* Initial respiration rate—; 15; about 0.1 cmm. O_2/cc./hour. Final pH 7.0 ±0.1; 7.1 ±0.1; 6.8 ±0.1. Source of nitrogen: N_2; 50 p.p.m. NO_3-N; N_2. VI/26–27/30; VII/18/30; VIII/7–10/30.

* For explanation of this phenomenon see 11, "length of the induction period." It has been found empirically that in general no humic acid stimulation is observed until the cultures have attained a heaviness of growth corresponding to a respiration rate of about 5 or more cubic millimeters O_2 per cubic centimeter per hour.

† Accurate to ± 1 cmm.

We do not wish to be understood as precluding the possibility of humic acid stimulation of pure cultures of Chlorella under conditions other than we have employed, even though Clark (13, 15) also obtains stimulation of Lemna only in non-sterile medium but not in sterile medium. The universally obtained

TABLE 20

The absence of stimulation of growth of pure cultures of Chlorella by humic acid or iron compounds under specific conditions

		EXPERIMENT 20		EXPERIMENT 20A
Duration (hours)		142		118
Initial concentration cells (millions per cubic centimeter)		0.066*		0.23*
Final pH		6.9		6.7
Substance added	Concentration	Final dry weight† (mgm. dry matter per 100 cc.) (absolute growth)	Final net turbidity (relative growth)	Final net turbidity (relative growth)
	p.p.m.			
None	.....	60.0	100 ± 5	100 ± 15
Citric acid (neutralized to pH 7)	250	66.7	100 ± 5	....
	100	....	100 ± 5	....
	25	....	100 ± 5	100 ± 15
	12.5	....	100 ± 5	....
	2.5	....	100 ± 5	....
	0.5	....	100 ± 5	....
Fe-citrate	100	60.0	100 ± 5	....
	25	....	100 ± 5	100 ± 15
	12.5	....	100 ± 5	....
	2.5	....	100 ± 5	....
	0.5	....	100 ± 5	....
$Fe_2(SO_4)_3$	20	65.2	100 ± 5	....
	5	....	100 ± 5	100 ± 15
	2.5	....	100 ± 5	....
	0.5	....	100 ± 5	....
H. A.-I.	100	62.3	80 ± 5	100 ± 15
	25	....	100 ± 5	100 ± 15
	12.5	....	100 ± 5	....
	2.5	....	100 ± 5	100 ± 15
$Fe_2(SO_4)_3$ plus H. A.-I.	5	....	....	....
	25	....	....	100 ± 15
$Fe_2(SO_4)_3$ plus neutralized citric acid	5	....	....	....
	25	....	....	100 ± 15

* The final concentration of cells (determined with a haemacytometer) was about 6 million per cubic centimeter, in all cases in both experiments, corresponding to a generation time of about 25 hours.

† The dry weight measurements represent a composite determination, in the case of any given compound, of all the respective concentrations, and were made by combining 25-cc. portions from each flask of each concentration of a given compound and centrifuging the cells, transferring the cell precipitates to small weighed centrifuge cups, recentrifuging, decanting the clear liquid, and reweighing after drying *in vacuo* at room temperature. The variability of only 5 per cent confirms the turbidity measurements.

The pure cultures of Chlorella were grown under much the same conditions employed by Warburg (52). The medium consisted of 1,000 cc. of H_2O containing 2.46 gm. $MgSO_4 \cdot 7H_2O$, 1.22 gm. KH_2PO_4, 1.26 gm. KNO_3, and 0.0042 gm. $Fe_2(SO_4)_3 \cdot 9H_2O$. Under the conditions employed growth was unaffected by the presence or absence of $CaCO_3$. 50 cc. sterile medium were contained in each 500-cc. rubber-stoppered flask. The flasks were aerated in series with washed air containing 5 per cent CO_2 (the optimum CO_2-content), and were vertically illuminated at a distance of 34 inches by 100-watt Mazda frosted lamps so arranged that substantially uniform illumination was obtained. The temperature was 23–25°C. Cultures were run in duplicate.

stimulation of growth of impure cultures of the lower green plants by organic extracts probably takes place, or is induced, as a result of the relatively more rapid depletion of *soluble* iron from the medium caused by bacteria or other similar contaminating forms, whose iron requirements are presumably higher. Consequently, if stimulation of pure cultures is desired, care must be taken to eliminate iron from the culture medium or that already present as impurity will generally suffice to prevent stimulation by iron bound with organic matter intentionally added. Cultures with low iron requirements, as compared to those with high requirements, can undoubtedly be relatively more easily accommodated by the slowly and very slightly soluble, inorganic iron. It is probable that pure cultures grown for several transfers in a medium as free from iron as possible would submit to stimulation.

TABLE 21

The influence of Ca-humate upon the growth of Azotobacter in a calcium-deficient medium (Erlenmeyer technique)

	CALCIUM FREE* CUSTOMARY MEDIUM PLUS SOLUBLE Ca AS						
	Nothing	$CaSO_4 \cdot 2H_2O$	Ca-humate†				H. A.-VII (200 p.p.m.) (no Ca)
Concentration of soluble calcium added (p.p.m.)	.	15	2.4	9.4	24	48	..
Final count (millions per cubic centimeters)	6	50	25	35	45	50	8
Per cent growth increase	.	730	315	480	650	730	33

Inoculated with 1 drop of 5-hour old *A. vinelandii.* Analyzed after 4 days. Final pH 6.9 ±0.1. Source of nitrogen: N_2.

* Prepared by omitting the customary 100 p.p.m. $CaSO_4 \cdot 2H_2O$.

† A synthetic preparation containing 4.7 per cent Ca and less than 0.03 per cent Fe. In the presence of 100 p.p.m. $CaSO_4 \cdot 2H_2O$ this iron-poor Ca-humate preparation has substantially no stimulative action.

It is realized that various humic acids, prepared from various soils or sugars under various conditions, might conceivably exert their stimulating effects from various causes, i.e., from contained iron, contained aluminum, contained silica. As shown in table 21, when some essential element, in this instance calcium, is omitted from the culture medium of Azotobacter, then humic acid containing that element may supply it in a form which is comparable although not superior in availability to the form ordinarily supplied. As a matter of fact, indeed, because of the high molecular weight of humic acid, the Ca-humate (4.7 per cent Ca) is less effective per unit dry weight of humic acid than $CaSO_4 \cdot 2H_2O$. The H. A.-VII culture acts as a control on the Ca-humate cultures in showing that a calcium-free synthetic iron-containing humic acid can not replace an iron-free synthetic calcium-containing humic acid in a calcium-free medium. All that can be reasonably claimed for the data of this paper is that contained iron is probably the chief cause of humic acid growth stimulation.

DISCUSSION

Our chief endeavor has been to prove or to establish, so far as possible, the chemical nature of humic acid stimulation, rather than to indicate a few suggestive and limited experiments in connection therewith. We have therefore not hesitated to present in this paper and in the following paper (11) a comprehensive body of experimental material. It should easily be evident that this procedure is essential, because of the manifold factors which must be studied before any satisfactory conclusion or conclusions can be reached concerning the cause or mode of operation of humic acid stimulation. We cannot emphasize too strongly this general consideration.

It is obviously impossible, in this long report, to give more than an indication of the broader implications of these findings and to make reference to the most pertinent recent literature (2, 3, 4, 6, 12, 13, 14, 15, 16, 18, 19, 20, 21, 23, 24, 32, 33, 34, 35, 36, 37, 38, 41, 43, 44b, 46, 49, 51, 54, 56, and works cited therein). It is believed that these findings relate to various fields outside the immediate scope of this paper, as follow: The chemical constitution of the active components of the numerous growth-promoters or essential nutrients such as yeast-water, plant extract, peat extract, soil extract, bios, auximones, egg albumen, egg extract, Rhizopin, and vitamin B; the substitution of certain of these substances of unknown general chemical constitution by synthetic iron-containing humic acids, in the preparation of growth media; the iron nutrition of higher green plants grown in water cultures; the influence of soil humus and organic matter upon soil fertility, as a function of iron supply. It would be desirable to study plants with high requirements for certain less common elements, such as Al, Mn, Cu, or B, and determine whether these could be supplied especially readily in the form of humates. In regard to iron humates, further work would be desirable in preparing natural or synthetic humic acids with in general still shorter induction periods, or greater effectiveness per unit of dry weight of humic acid (i.e., with still higher iron contents). This could be done by separating humic acids into each of their various organic acid constituents and then selecting, if there were any differentiation, the most superior, especially those compounds of low molecular weight. Finally, there remains the fundamental question of why humic acid iron is in general so much more available than the other types, at least those so far studied; at present we incline to the view that the explanation lies in the adsorbed condition of the humic acid iron (27) and its high solubility. In this connection study might be made of other colloidal carriers of iron besides the humic acids. All these problems yet to be solved should not be confused with the chief problem brought to solution in this paper, namely, the establishing of the rôle of iron in the stimulation of growth by humic acid.

SUMMARY

Upon the basis of a number of independent lines of evidence it is shown that humic acid causes stimulation of Azotobacter growth primarily by virtue

of its *iron* component; other inorganic impurities and the organic fraction are substantially inactive.

Natural humic acid may be substituted for more or less satisfactorily by (*a*) complex, non-ionized organic acid iron such as ferric citrate, tartrate, oxalate; (*b*) inorganic iron such as ferric sulfate, metallic iron; (*c*) humic acids prepared synthetically from glucose and ferric sulfate, and containing considerable quantities of iron.

Natural humic acid was not substituted by (*a*) humic acids prepared synthetically from glucose to contain no iron, or as little iron as possible; (*b*) iron-free synthetic humic acids containing various metals such as Al, Co, Mn, Si, Mo, and P; (*c*) salts of such elements as Al, Co, Mn, Mo, Ni, Zn, Cr, Cu, and Ag at various metal concentrations ranging from 0.01 to 50 p.p.m.

The activity of a natural humic acid, per unit of dry weight, may be increased by synthetically increasing its iron content.

Natural and synthetic iron-containing humic acids have been shown to alleviate a specific case of iron-deficiency occurring under certain conditions of nitrogen fixation.

Under conditions where humic acid exerted no stimulative effect the iron substitution compounds likewise exerted no effect, even though other chemical substances and physiological conditions could at the same time increase the growth rate considerably.

A statistical study of all the available data shows that, from a quantitative standpoint, *a given sub-optimal amount of soluble iron (about 0.2 to 1 p.p.m. or less) gives the same order of magnitude of increased growth, independently of whether the iron is added as contained in natural humic acid or in any of its substitution compounds.* This statement requires the qualification that the experiments must proceed long enough to avoid limitation through induction period differences and to allow complete consumption of the sub-optimal physiologically active iron added. In the case of Azotobacter, natural humic acids commence to exert stimulation some hours sooner than organic acid iron compounds, and the latter again somewhat sooner than inorganic iron compounds; the synthetic iron-containing humic acids behave substantially as the natural ones. Consequently, *in experiments of relatively short duration it will often be found that inorganic iron, and even organic acid iron, will provide considerably less satisfactory stimulation than iron contained in natural or synthetic humic acids.*

In view of this work, humic acid is to be classed as both a nutrient which provides iron for growth and nutrition, and a stimulant which provides iron in a form in general *more highly available* than that usually supplied in the medium. Humic acid is not a plant-vitamin, or any other constituent classifiable as *essential* to the medium, since it may be replaced by other substances containing iron.

Like many other inorganic and organic compounds of calcium, synthetically prepared calcium humate may supply calcium to Azotobacter in a calcium-free medium, but in a form *no more available* than that usually supplied in the medium (calcium sulfate). Other similarly incomplete media could undoubtedly be improved in a similar manner by humates containing the respective lacking element, but this action would be a matter simply of nutrition and not of increased availability, and relatively large concentrations of the humates would have to be employed.

Strictly interpreted, the reported findings apply only with respect to Azotobacter; but many reasons obtain for believing them to be of widespread application. Even though humic acid might with certain organisms and under certain special circumstances, play some rôle other than here assigned, the fact that in any case it is capable of increasing the availablity of iron would seem established beyond question. Humic acid is probably one of the few growth-promotors whose determining constitution has been thus established.

Upon the basis of this work explanations in part are offered for (*a*) the widely different amounts of nitrogen fixed by Azotobacter per gram of sugar consumed, when time is not a variable; (*b*) Clark's finding that growth stimulation of certain lower green plants by organic extracts occurs only in the presence of contaminating organisms; (*c*) the nature of the chemical constitution of various growth-promoters; and (*d*) the beneficial influence of humus and organic matter upon soil fertility.

Postscript added to galley proof. Since submission of the manuscript of this paper to press, Dr. Earl S. Johnston, division of radiation and organisms, Smithsonian Institution, Washington, D. C., has very kindly performed some experiments on the effect of natural and synthetic humic acids upon the growth of tomato plants in water cultures under artificially maintained conditions of temperature, humidity, and lighting, and also under greenhouse conditions. The following findings appear reasonably certain. A single initial dosage of 0.5 to 5 p.p.m. iron as iron humate provides iron in a form equal to, or superior to, dosages of 1 to 5 p.p.m. iron as iron tartrate added bi-daily throughout the growing period, the comparisons being made upon the basis of green and dry weights of tops and roots, organic nitrogen content, thickness of stems, extent of foliage, number of rootlets, and green coloring. The culture solutions containing iron humate do not accumulate the large precipitates of iron phosphate obtained with the customary forms of iron added, such as iron tartrate or sulfate. The concentration functions of the natural and synthetic iron humates with respect to both iron and humate fractions are substantially the same as those given by Azotobacter [figs. 2 and 3 and (11, fig. 2)]; synthetic humic acids containing 5 to 10 per cent Fe are required in smaller concentrations than the natural humic acids containing 1 per cent Fe; independently of the iron, the synthetic humate fraction becomes toxic at about 100 p.p.m. (as determined by using synthetic humic acids containing low contents of iron), whereas much higher concentrations of natural humic acids are required for toxicity by the humate fraction. More iron per unit of dry weight is required by Azotobacter than by tomato plants, but less dry weight is produced per unit volume of culture solution, so that the actual iron requirements per unit of culture solution are about the same. On the whole, the synthetic humic acids are probably somewhat preferable to the natural ones, in view of their better known composition, although where traces of unknown elements, and not merely iron, are required, the natural acids may be preferable. In the experiments being reported upon, no appreciable growth took place unless iron in some form were added. Thus, in one experiment lasting 3 weeks, the dead withered seedlings which resulted in the case of the solutions to which no iron was added weighed 0.08 gm. (dry weight), compared to 1.22 and 2.51 gm. given by 0.02 and 2.0 p.p.m. iron as synthetic iron humate, or 2.24 gm. given by 11 p.p.m. iron as iron tartrate added in 11 bi-daily dosages of 2 p.p.m. iron each.

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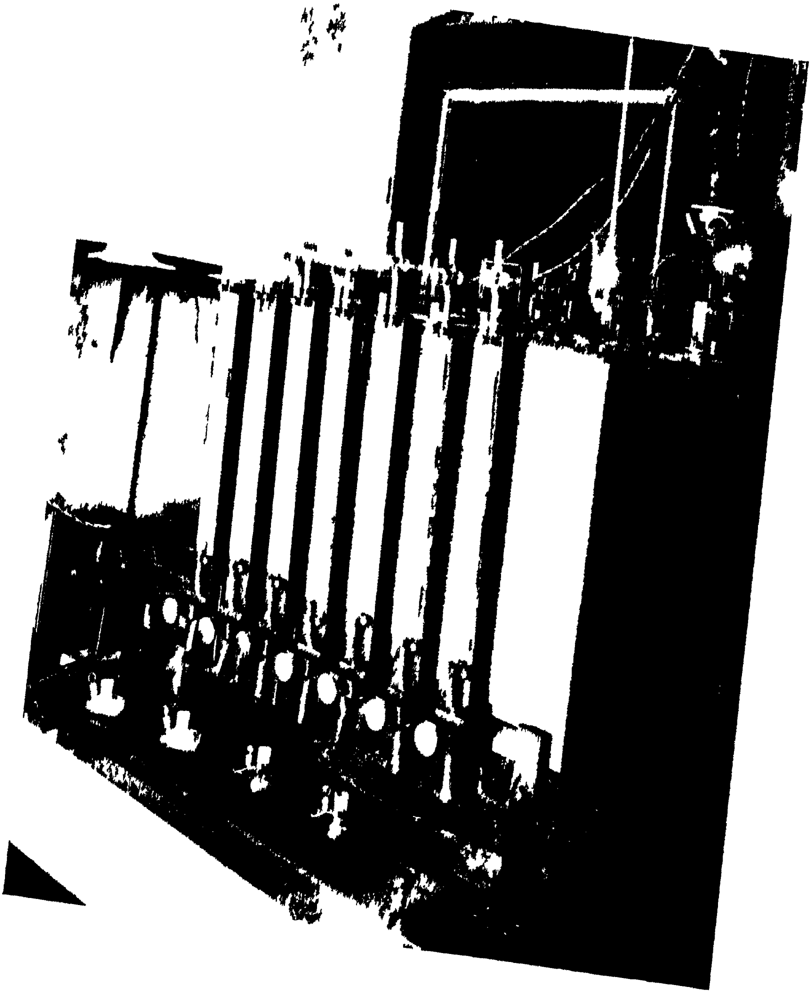
PLATE 1

WARBURG MICRO-RESPIRATION APPARATUS FOR MEASURING OXYGEN CONSUMPTION AND GROWTH

The cultures are contained in vessels (see figure 1) submerged in the constant temperature water bath. The vessels (not visible) are attached to the manometers, which are shaken at the rate of 120 cycles per minute with an amplitude of 3 cm. Fourteen manometers are accommodated in the apparatus, making a large number of simultaneous experiments possible. The temperature of the thermostat is generally maintained at 28° ±0.01°C. or 31° ±0.01°C.

STIMULATION OF GROWTH BY HUMIC ACID
D BURK, H LINEWEAVER AND C K HORNER

PLATE 1



THE PHYSIOLOGICAL NATURE OF HUMIC ACID STIMULATION OF AZOTOBACTER GROWTH

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This paper will present an analysis of various physiological aspects of stimulation of growth of Azotobacter caused by humic acid and substances capable of substituting for humic acid. Emphasis will be laid chiefly upon the relations obtaining immediately subsequent to application of the stimulant. Apart from our previous work reported casually in other connections (2, 3, 4, 5, 8), and the work of Iwasaki (11), little attention has been paid to these dynamic, differential relations. Most other investigations, including our own preceding one (8), have been concerned experimentally chiefly with effecting by means of humic acid large integral increases of growth in cultures grown for days or weeks. Functional relationships based upon velocities obtained in this manner are subject, however, to the contingent disadvantage that the duration of the experiment is a variable and often uncontrolled factor involving secondary incidental phenomena (2, p. 1177; 4, p. 390). In order to eliminate this objectionable feature, the velocity *constant* of growth will frequently be employed here, in preference to the velocity of growth. Correspondingly, the "humic acid velocity constant ratio" will be employed in preference to the "humic acid ratio," as a quantitative criterion of stimulation. Since growth in young cultures of Azotobacter maintained under constant conditions is logarithmic, functions based upon the former ratio will be independent of time factors, whereas functions based upon the latter ratio will vary with time (see table 1 and discussion thereof, "The Theoretical Integral Function").

EXPERIMENTAL METHOD

The preparation and description of the various humic acids employed, and the general experimental procedure, are described in the preceding paper (8). It may be recalled that the monomolecular velocity constant of growth is obtained by plotting natural logarithm of growth with respect to time, which yields a line the slope of which represents the constant g in the formula "$g = d\ ln\ (a + y)/d\ t$", where a is the initial amount of inoculum and y is the growth increase after t, the time in hours; g is an important fundamental criterion of growth since it represents *at all times* the rate of growth at unit concentration of cells, as distinguished from the observed velocity, which varies with the concentration and therefore with the time. (For further discussion of the velocity constant g, see 6, 14.) The g values reported in this paper will be based upon not one, but several values of y at respective t values, in order to average out minor experimental errors causing apparent departures of growth from being strictly logarithmic. The terms $g_{H.A.}$ and g_0 will refer to the velocity constants of growth in the presence and absence of humic acid (or substitutes of humic acid) respectively, the ratio between the two being the humic acid velocity constant ratio (H. A.-V. C. R.).

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THE HUMIC ACID CONCENTRATION FUNCTIONS

The differential functions

The concentration of natural humic acids yielding approximately optimum stimulation varies from 25 to 100 p.p.m., 90–100 per cent of the optimum being obtained most frequently at about 50 p.p.m., as shown in figure 1. This statement applies to experiments so conducted that significant decreases in the concentrations of the active fraction of humic acid do not occur during the period of measurement, i.e., the humic acid velocity constant ratio remains constant with respect to time, for any given concentration. Functions so obtained represent experiments in which the amount of growth measured is in effect infinitesimally small, since the time is also, in effect, infinitesimally small, and consequently they have been termed "differential." The integral functions represent the converse situation, where growths and durations are finite.

The corresponding concentration of iron contained in humic acid, yielding optimum stimulation, is about 0.2–0.8 p.p.m. The synthetic humic acids may be prepared to contain at least two to three times the amount of iron found in our preparations of iron-rich natural humic acids (0.75–1.5 per cent), and the "differential" optimum concentration is correspondingly lower, 5–25 p.p.m. (see fig. 3A).

The natural humic acid differential concentration function passes through a maximum at 200–600 p.p.m., as may be gathered from figure 2, curve V, and the corresponding synthetic humic acid function is correspondingly lower, about 100–300 p.p.m. We are of the opinion that the decreased stimulation occurring at higher concentrations is not due chiefly to iron, but for the most part to the organic fraction of the humic acid, inasmuch as synthetic humic acids containing practically no iron behave somewhat similarly.

Because of solubility limitations, it is ordinarily impossible to attain a concentration of inorganic iron sufficient to yield a differential optimum concentration, but the theoretical value is probably about 0.5 p.p.m. of *soluble* inorganic iron.

As may be gathered from fig. 3B, curve 4E (and to some extent from curves 5, 7B) the implied differential optimum concentration of ferric citrate is 2.5 p.p.m., or about 0.5 p.p.m. ferric citrate iron.

As seen in figure 1, the optimum H. A.-velocity constant ratios vary, in the case of young vigorous cultures, from 1.25 to 1.50. This has been our experience also in a large number of miscellaneous unreported experiments. The ratio may become somewhat higher at times, but only when the g value at 0 p.p.m. H. A. is very low, about 0.08 or less, corresponding to a generation time of about 8 or more (see "Constant Temperature Conditions"). It will be noticed that the various curves in figure 1 are in general higher as the g value at 0 p.p.m. is lower, i.e., an inverse function obtains. This fact is highly

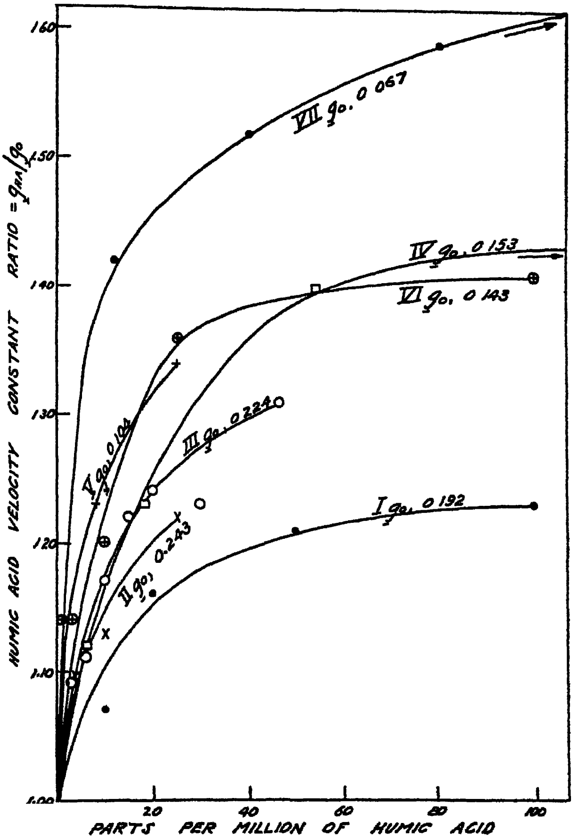

FIG. 1. THE DIFFERENTIAL INFLUENCE OF CONCENTRATION OF NATURAL HUMIC ACID UPON THE HUMIC ACID VELOCITY CONSTANT RATIO (WARBURG TECHNIQUE)

The g values in the presence and absence of humic acid, used to obtain the H. A.-Velocity Constant Ratio, are based in every case upon hourly y and t values observed over a period of 8–15 hours. g_0, used in denoting the curves, refers to the g value at 0 p.p.m. humic acid. All experiments performed at 28°C. Curves I, II, III, IV, V, VI, VII, cultures aged, in days, respectively 1, 1, $\frac{3}{4}$, 1 (diluted 2 times), 1, 1, 4 (diluted 6 times); initial respiration (cmm. O_2/cc./hour) respectively 4, 20, 20, 8, 25, 25, 65. H. A.-I employed in all cases, except H. A.-II in curve V. Source of nitrogen: N_2, except in curves II and III, where 50 p.p.m. NH_3-N.

significant and is commented upon further in the section "The Influence of Velocity of Growth in the Absence of Humic Acid upon the Humic Acid Velocity Constant Ratio."

The integral functions

As the physiologically active fraction of the humic acid is consumed during the time course of an experiment, the observed "integral" optimum concentra-

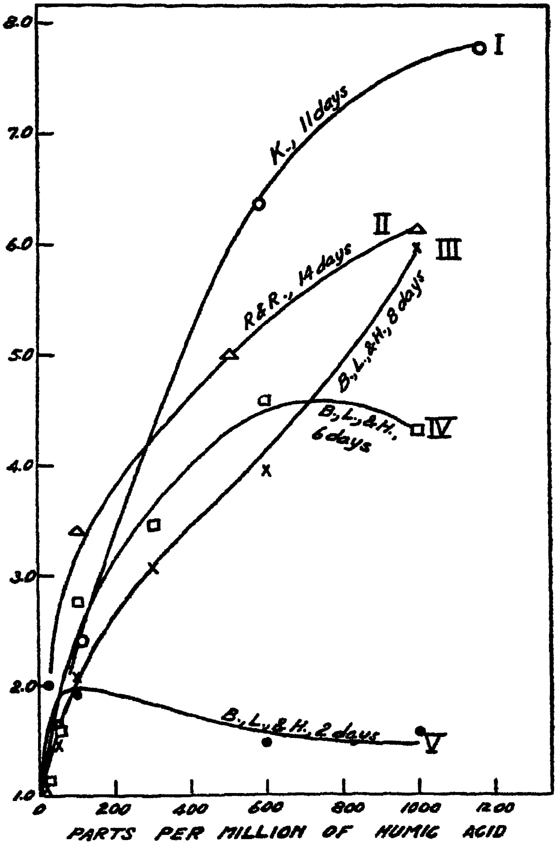

Fig. 2. The Integral Influence of Concentration of Natural Humic Acid upon the Humic Acid Ratio in Experiments of Very Long Duration (Erlenmeyer Technique)

Curve I. Data of Krzemieniewsky (13, p. 1004). Cultures grown in 200 cc. 1½ per cent glucose medium in large flasks. Growth measured as nitrogen fixed. Source of nitrogen: N_2. (The concentration of humic acid refers, in this single instance, to potassium humate.)

Curve II. Data of Remy and Rösing (15, p. 354). Cultures grown in 100 cc. 2 per cent mannite medium in large flasks. Growth measured as nitrogen fixed. Source of nitrogen: N_2.

Curve III, IV, and V. Data of Burk, Lineweaver, and Horner. Respectively 2, 6, and 8-day old cultures of the same experiment. H. A.-V employed. Each point represents an average of duplicates. 2 per cent glucose employed instead of 1 per cent (in anticipation of the length of the experiment). Final pH: 6.8 ± 0.1. Source of nitrogen: 200 p.p.m. NO_3-N.

tion will obviously shift higher and higher. When completely consumed, and the logarithmic phase of growth has been passed through, the growth velocity constant gradually becomes relatively less, per unit of cell concentration, compared to the control, as a result of consumption of the necessary elements of the medium, viz., Ca, P, Mg, glucose, not to mention Fe.

Figure 2 shows that in experiments of several days or weeks duration the integral optimum concentration may increase to 1,000–1,500 (and –2,000) p.p.m. natural humic acid. It is this type of concentration function which is usually reported in the literature; figure 2 represents exemplary data of other investigations [Krzemieniewsky (13), Remy and Rösing (15)], in addition to our own, which, it will be observed, are very similar. It is obvious that in representing integral functions for cultures several days old the humic acid ratio and not the velocity constant ratio is to be used.

Curves V, IV, and III of figure 2 represent a continuous experiment and show excellently the shifting of the integral optimum concentration with time. The optimum values are 50–300, 600–800, and greater than 1,000 (probably 1,500) p.p.m. at respectively 2, 6, and 8 days growth. Even the upward concavity developing in curve III is typical. It occurs because in relatively long-time experiments, because of limitation by other secondary factors, the humic acid ratio as a function of time passes through a maximum for sub-optimal amounts of humic acid [see (8, table 15)]. This effect is relatively greater the smaller the concentration. The general result is that the sub-optimal portion of the integral humic acid ratio concentration function changes with time from being first concave downward, to being relatively linear, to being finally concave upward, when the cultures are grown in unshaken flasks and no renewal of the medium is made. It will be noticed in this connection that, as might be expected, curve III lies, for the most part, below curve IV, even though the latter represents a younger culture. The actual growths in the case of curve III are, of course, greater; only the humic acid ratios are smaller, having been calculated, as usual, upon the basis of the growth in the 0 p.p.m.-culture for the respective day.

The integral concentration functions of synthetic humic acids and organic and inorganic iron behave qualitatively much the same as those already described in connection with natural humic acid. Comparing, for instance, in figure 3A, curves B, 11A, and those remaining, one notes that the synthetic humic acid optimum shifts from about 5 to 10, 30 to 50, to greater than 60 p.p.m., at 13 hours, 18 hours, and 2–11 days, respectively.

Mixed functions

In figures 3, 3A, and 3B are represented a number of miscellaneous experiments, including all the appertaining ones reported in the preceding paper (8). These will provide additional evidence with respect to the various relations discussed. In general, the experiments represented are of an intermediate type. They have been conducted for a period of time either somewhat too long or somewhat too short, respectively, to demonstrate either the minimum

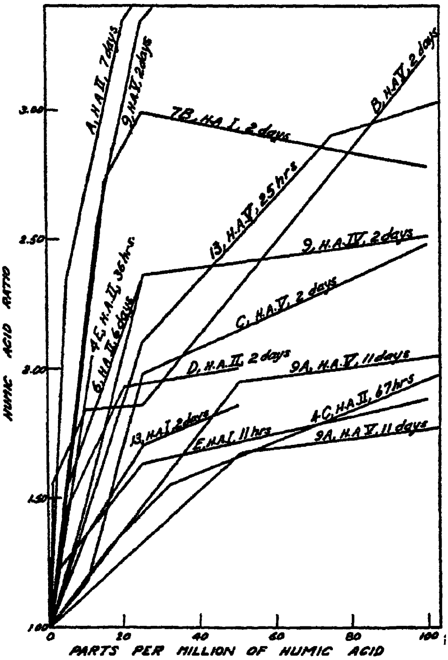

Fig. 3

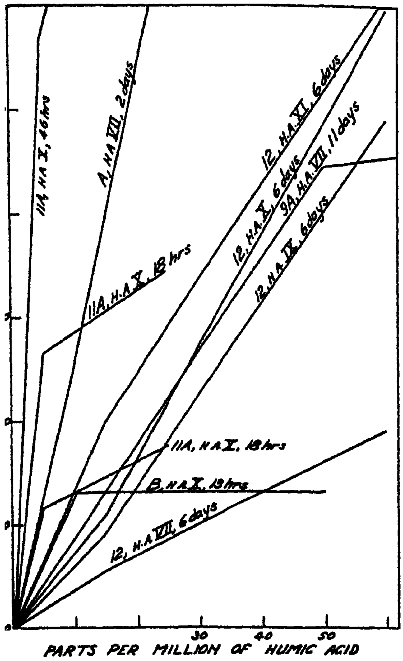

Fig. 3a

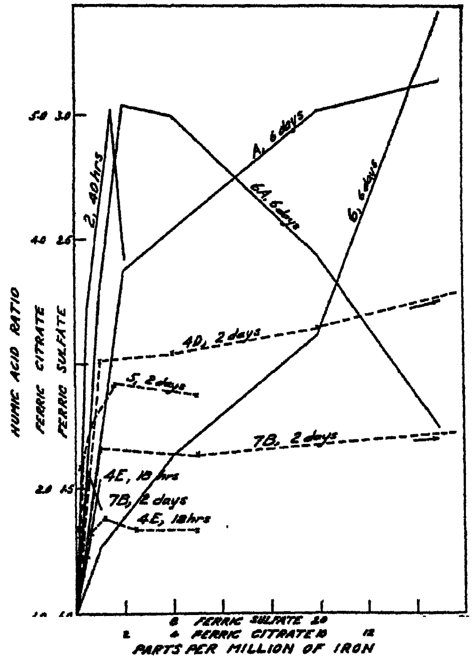

Fig. 3b

differential optimum concentration or the maximum integral optimum concentration.

Experiment 9A in figures 3 and 3A is possibly exceptional in being still concave downward, but since 2 per cent glucose was not employed in this very long experiment the energy supply must have imposed an important limiting secondary factor preventing the higher humic acid concentrations from exerting their usual effect. The last point on curve 7B (100 p.p.m.) is probably low. The difference of behavior of curves A, 6, and 6A, fig. 3B, all representing 6-day experiments with inorganic iron, illustrates the considerable latitude of variation which must often be expected in the exact time relations of relatively old cultures.

The theoretical integral function

It is evident from figures 2, 3, 3A, and 3B that the integral humic acid ratio rarely exceeds 8 to 10, even in long-time experiments, and that, in experiments of not more than a week's duration, values of 2 to 4 prevail as a rule. Were it not for such secondary factors as those discussed in the section "The Integral Functions", viz., consumptions of active fractions of humic acid, inorganic nutrients, energy supply, etc., much higher ratios might be expected, as shown in table 1. This is true in spite of the additional factor that a culture can obviously become only so heavy, through purely physical limitation.

In table 1 is represented the theoretical humic acid ratios which would obtain if by some means physiological consumption factors and physically limiting factors could be eliminated, and optimum humic acid velocity constant ratios could be maintained indefinitely. Guided by figure 1, we have given values for three different ratios, covering the normal range, 1.25, 1.50, and 1.75. Values are also given, in the case of each ratio, for three different velocity constants at 0 p.p.m. H. A., 0.175, 0.116, and 0.086. In order of magnitude the first two constants occur most frequently in *Warburg* technique

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FIG. 3. THE MIXED INTEGRAL-DIFFERENTIAL INFLUENCE OF CONCENTRATION OF NATURAL HUMIC ACID UPON THE HUMIC ACID RATIO

Curves A, B, C, D, represent turbidity measurements. Curve E represents respiration rate measurements and is taken from the same experiment reported in figure 1, curve VI. The other curves correspond to respective tabular data in the preceding paper (8).

FIG. 3A. THE MIXED INTEGRAL-DIFFERENTIAL INFLUENCE OF CONCENTRATION OF SYNTHETIC IRON-CONTAINING HUMIC ACID UPON THE HUMIC ACID RATIO

Curves A and B represent respiration rate measurements. The other curves correspond to respective tabular data in the preceding paper (8).

FIG. 3B. THE MIXED INTEGRAL-DIFFERENTIAL INFLUENCE OF CONCENTRATION OF INORGANIC AND ORGANIC ACID IRON UPON THE HUMIC ACID RATIO

Solid lines, ferric sulfate; broken lines, ferric citrate. Curve A represents turbidity measurements. The other curves correspond to respective tabular data in the preceding paper (8). To convert abscissal values to p.p.m. of iron "compound" multiply by 5.

experiments, the latter two in *Erlenmeyer* technique experiments, at 28° – 31°C. It is seen that even after only about 24 hours ratios greater than 8 to 10 may be expected, becoming 20 to 60 after 2 days, and attaining absurd values in 3 to 5 days or more.

Table 1 illustrates the relation already mentioned, that when the H. A.-velocity constant ratio is kept constant with respect to time the H. A.-ratio increases very rapidly with time, especially after about 12 hours, the increase being logarithmic. It is also true that for a given period of time and for a given H. A.-velocity constant ratio the H. A.-ratio likewise increases logarithmically with the magnitude of g_0.

TABLE 1

The theoretical humic acid ratio as a function of time, at a maintained optimum constant humic acid velocity constant ratio

	H. A.-V. C. RATIO								
	1.25			1.50			1.75		
Generation time (G. T.) (in absence of H. A.).	8	6	4	8	6	4	8	6	4
Corresponding velocity constant (in absence of H. A.).	0.086	0.116	0.175	0.086	0.116	0.175	0.086	0.116	0.175
Theoretical humic acid ratio									
Time, *hours* 0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
4	1.09	1.12	1.19	1.19	1.26	1.42	1.29	1.41	1.69
8	1.19	1.26	1.42	1.41	1.58	2.02	1.67	2.00	2.85
12	1.29	1.41	1.69	1.67	2.00	2.87	2.15	2.82	4.80
24	1.67	2.00	2.86	2.78	3.98	8.13	4.63	7.94	23.2
48 (2 days)	2.78	3.98	8.13	7.73	15.8	66.8	21.5	63.1	537
120 (5 days)	12.9	31.6	191	167	200	355	2,090	3×10^4	6×10^6
168 (1 week)	35.8	126	1,560	1,280	2×10^6	2×10^6	5×10^4	2×10^6	3×10^9
336 (2 weeks)	1,280	2×10^4	2×10^6	2×10^6	3×10^9	6×10^{12}	2×10^9	4×10^{12}	1×10^{19}

THE INFLUENCE OF VELOCITY OF GROWTH IN THE ABSENCE OF HUMIC ACID UPON THE HUMIC ACID VELOCITY CONSTANT RATIO

Constant temperature conditions

It has been shown (8) that humic acid is a stimulant but not an essential for growth, and that it adds iron in a form more soluble and highly available than the usual inorganic iron supplied. It would seem reasonable to expect, therefore, that as the growth velocity is increased, from one cause or another, humic acid stimulation will decrease, since there is less opportunity for stimulation; a maximum possible velocity is being approached which is determined by numerous other factors (glucose concentration, salt concentration, temperature, etc.) which are, on the whole, unrelated to the stimulating agent. At low velocities the ratio (maximum possible velocity)/(low velocity) is much higher than at high velocities and opportunity for stimulation presumably much greater.

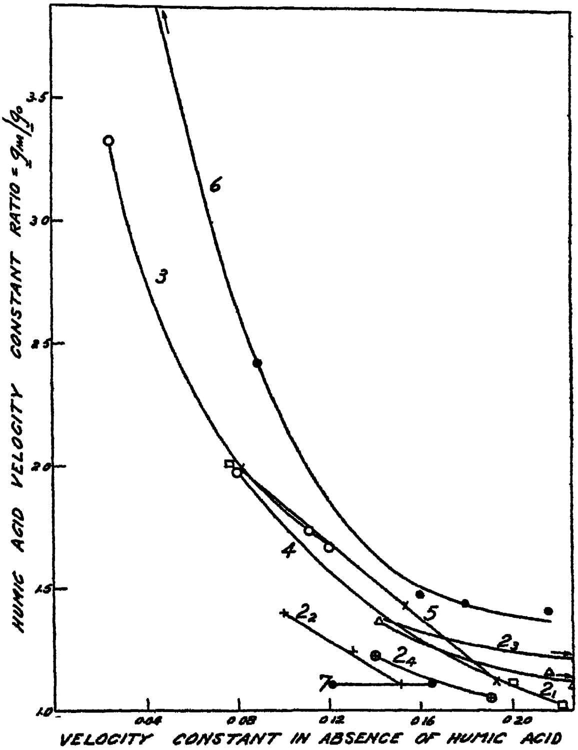

FIG. 4. STIMULATION AS A FUNCTION OF GROWTH VELOCITY
The curves are lettered corresponding to the data given in tables 2–7 inclusive

The inverse relationship between growth velocity and stimulation is obtained, in fact, with respect to all factors regulating growth velocity under conditions of constant temperature, as follow: nitrogen source; pressure of nitrogen gas; concentration of fixed nitrogen; medium constitution; and age or heaviness of culture; as shown, respectively, in tables 2, 4, and 5; 3; 4; 5; and 6. Figure 4 presents a plot of all the functions.

The close similarity in form and position of the curves in figure 4 is somewhat surprising. They all fall between the limits of a narrow two-dimensional hyperbola which tends to approach the abscissa and ordinate practically within experimental error of measurement at, respectively, g_o = approximately 0.2, and H. A.-velocity constant ratio = approximately 5. It would appear that the latter ratio rarely exceeds 1.15 – 1.20 when the velocity constant in the absence of humic acid exceeds 0.14, from whatever cause. Conversely, the ratio generally exceeds 2.00 when the constant falls below 0.08 to 0.10, and increases still further very rapidly as the constant becomes still smaller.

TABLE 2

The influence of growth velocity (as governed by nitrogen source) upon humic acid stimulation (Warburg technique)

Experiment 1:				
Relative velocity constant (in absence of H. A.)	1.00	1.35	1.41	1.55
Source of nitrogen (fixed nitrogen)	N_2	NO_3-N (50)	NH_3-N (50)	Urea-N (50)
g, no humic acid	0.160	1.216	0.226	0.248
g, 50 p.p.m. H. A.-V	0.202	0.248	0.250	0.258
H. A.-velocity constant ratio	1.26	1.15	1.10	1.04
Experiment 2:				
Relative velocity constant (in-absence of H. A.)	1.00	1.30	1.50	.....
Source of nitrogen (fixed nitrogen: p.p.m.)	N_2	NO_3-N (50)	NH_3-N (100)	.....
g, no humic acid	0.100	0.130	0.150	.....
g, 25 p.p.m. H. A.-II	0.140	0.160	0.165	.....
H. A.-velocity constant ratio	1.40	1.24	1.10	.....
Experiment 3:				
Relative velocity constant (in absence of H. A.)	1.00	1.70	.....	.....
Source of nitrogen (fixed nitrogen: p.p.m.)	N_2	NH_3-N (50)	.....	.....
g, no humic acid	0.143	0.243	.....	.....
g, 25 p.p.m. H. A.-II	0.195	0.296	.....	.....
H. A.-velocity constant ratio	1.36	1.22	.....	.....
Experiment 4:				
Relative velocity constant (in absence of H. A.)	1.00	1.38	.....	.....
Source of nitrogen (fixed nitrogen: p.p.m.)	N_2	NO_3-N (50)	.....	.....
g, no humic acid	0.140	0.193	.....	.....
g, 40 p.p.m. H. A.-I	0.172	0.203	.....	.....
H. A.-velocity constant ratio	1.23	1.05	.....	.....

Experiments 1, 2, 3, 4, respectively: 1, 1, 1, 2 days old; 31°, 27°, 28°, 28°C.; final pH 6.7, 7.0, 6.8, 7.0 ± 0.1 (except in NH_3-N cultures 6.6 ± 0.2).

In some, but not all, of the appertaining tables, somewhat larger stimulation is obtained in free than in fixed nitrogen, but this is what would be expected, quantitatively, in view of the correspondingly somewhat smaller growth velocity and also the differences between other forms of fixed nitrogen. There is no need to consider that humic acid is exerting any significant additional influence upon the mechanism of nitrogen fixation, although it may at times do so (6); the experiments of table 6 are possibly also an exception to this statement. As shown in table 4, when the growth velocity in nitrate-nitrogen

TABLE 3

The influence of growth velocity (as governed by nitrogen gas pressure) upon humic acid stimulation (Warburg technique)

Relative velocity constant (in absence of H. A.)	1.00	1.24	4.48	4.80
N_2 pressure (atm.)	0.10	0.25	0.45	0.78
g, no humic acid	0.025	0.081	0.112	0.120
g, 125 p.p.m. H. A.-II	0.083	0.160	0.195	0.200
H. A.-velocity constant ratio	3.32	1.97	1.74	1.66

Thirty-six-hour-old *A. chroococcum.* 0.20 atm. O_2 in different pressures of H_2 and N_2 to 1 atm. total pressure.

TABLE 4

The influence of growth velocity (as governed by supra-optimal concentration of fixed nitrogen) upon humic acid stimulation (Warburg technique)*

Relative velocity constant (in absence of H. A.)	2.91	2.63	1.00	(1.91)
Concentration of NO_3-N (p.p.m.)	625	1,250	5,000	(N_2-gas)
g, no humic acid	0.221	0.200	0.076	(0.145)
g, 50 p.p.m. H. A.-II	0.225	0.221	1.54	(0.199)
H. A.-velocity constant ratio	1.02	1.12	2.02	(1.37)

Eighteen-hour-old *A. vinelandii.* Final pH 7.0 ± 0.2.

* Supra-optimal rather than infra-optimal concentrations were employed—on account of the much greater ease in keeping the given concentrations constant throughout the course of the experiment.

TABLE 5

The influence of growth velocity (as governed by nature of medium) upon humic acid stimulation (Warburg technique)

Relative velocity constant (in absence of H. A.)	2.42	1.85	1.00
Source of nitrogen (fixed nitrogen: 50 p.p.m.)	NO_3-N	NO_3-N	N_2
Medium*	Customary	Phosphate	Customary
g, no humic acid	0.192	0.153	0.083
g, 50 p.p.m. H. A.-II	0.216	0.220	0.170
H. A.-velocity constant ratio	1.13	1.44	2.00

* Thirty-six-hour-old *A. vinelandii* diluted 6-fold with either customary (i.e., complete nutrient) medium or 0.1 per cent potassium phosphate (i.e., without Ca, Mg, Na, Fe, Cl, SO_4). 1 per cent glucose added to both media. Final pH 6.9 ± 0.1.

is lowered by excessive nitrate concentration to a value lower than the growth velocity in free nitrogen, the stimulation is then correspondingly greater. Moreover, the stimulation depends markedly upon the nitrogen pressure (table 3), and in table 5 stimulation in nitrate nitrogen is affected considerably by lowering the concentration of the common inorganic constituents, apart from potassium phosphate.

TABLE 6

The influence of growth velocity (as governed by heaviness of culture) upon humic acid stimulation (Warburg technique)

Relative velocity constant (in absence of H. A.)	7.2	6.0	5.3	2.9	1.00
Relative degree of heaviness of culture	1.0	2.5	3.1	4.4	7.5
Heaviness of culture (initial respiration rate) (cmm. O_2/cc./hour)	16	40	50	70	120
g,* no humic acid	0.216	0.180	0.160	0.089	0.030
g, 50 p.p.m. H. A.-V	0.302	0.258	0.236	0.215	0.136
H. A.-velocity constant ratio	1.40	1.43	1.48	2.42	4.53

Five 1-day-old *A. vinelandii* cultures which had been inoculated 24 hours previously with different amounts of inoculum so that after 24 hours different amounts of growth would be obtained. 31°C. Final pH 6.7 ± 0.1 Source of nitrogen: N_2.

* With the possible exception of the lightest, or two lightest, cultures, the growth velocities in the absence of humic acid were no longer strictly logarithmic, but tended to fall off gradually, chiefly because of incipient iron deficiency; consequently the average velocity for about second-eighth hour is employed. This procedure introduces only a slight uncertainty; the general conclusion is sufficiently established by this experiment, and many others not reported. Upon addition of humic acid, all cultures gave logarithmic growths. Whereas the differences in slope between the no-humic acid cultures are due chiefly to iron (and secondarily to other factors), the differences in the humic acid cultures are due chiefly to other factors (such as calcium, possibly phosphate, sugar, etc.)

TABLE 7

The influence of growth velocity (as governed by glucose concentration) upon humic acid stimulation (Warburg technique)*

Relative velocity constant (in absence of H. A.)	1.00	1.35
Initial glucose concentration (per cent)	0.1	1.1
g, no humic acid	0.122	0.165
g, 50 p.p.m. H. A.-V	0.132	0.178
H. A.-velocity constant ratio	1.11	1.11

* Data taken from table 11, fifth to thirteenth hour. Source of nitrogen: N_2.

It is seen in table 7 that although the growth velocity is 1.35 times as great in 1.1 per cent as compared to 0.1 per cent glucose, the H. A.-velocity constant ratio has not varied appreciably. Although it should not have been expected to vary markedly, in view of the magnitude of g and ratio values involved, approaching 0.14 and 1.0 – 1.1, respectively, an even more important circumstance lies in the fact that at the low glucose concentration energy supply (oxygen consumption) is rapidly becoming the chief limiting factor of growth and humic acid stimulation could not be expected in great degree.

Apart from tables 2–7, inclusive, and figure 4, further evidence in favor of the inverse relation between growth velocity and H. A.-velocity constant ratio may be obtained from figure 1 and figure 5, and from considerations of the effect of age of culture. In figure 1 the curves group themselves roughly in order of growth velocity in the absence of humic acid. In figure 5 stimulation is greater in nitrogen gas than in urea-nitrogen at all temperatures; at the same time the growth velocity is smaller at all temperatures (6, 14). We have observed that, in general, as the age of a culture increases the velocity constant decreases and the stimulation increases in much the same manner as shown in table 6, where differences in heaviness of culture are obtained by using different-sized inocula, rather than varying the age.

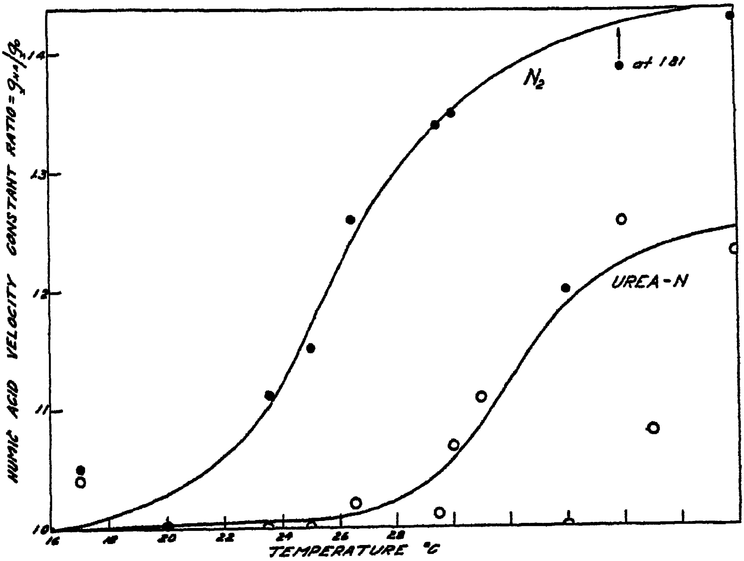

FIG. 5. THE INFLUENCE OF TEMPERATURE UPON THE HUMIC ACID-VELOCITY CONSTANT RATIO (WARBURG TECHNIQUE)

Iwasaki (11) has shown that as the oxygen pressure is lowered from 21 per cent to about 2–4 per cent, and the growth rate increases, the effect of humic acid decreases. This we have confirmed. Below 2–4 per cent oxygen the growth rate in the absence of humic acid again decreases, but humic acid has no effect here, nor in general does fixed nitrogen or other factors, since energy supply (oxygen consumption) is now more or less the completely limiting factor of growth rate.

The inverse relationship obtaining at any given constant temperature between growth velocity and stimulation may be interpreted as rather strong additional evidence that natural humic acid is to be classed as a stimulator of growth. Iron in *some* form is essential for growth, but the ability of natural humic acid to supply this iron is not specific. Another manner of regarding the matter is to consider that if iron is one of the chief factors controlling growth velocity, especially at the lower velocity, then the fact that one culture is growing more rapidly than another (with the same original iron supply) indicates empirically that less iron is needed in the former case and hence less stimulation could be produced.

The influence of temperature

The temperature function behaves to some extent conversely to the constant temperature growth velocity function just reported. Humic acid stimulation *increases* as the growth velocity is increased as a function of temperature over the *sub-optimal* growth velocity range (10° — 33°C. in N_2, and 10° — 33° — 37°C. in various forms of fixed nitrogen, see 6, 7, 14).

Figure 5 and table 8 present the data in connection with the influence of temperature upon the humic acid velocity constant ratio over the range 16° — 40°C. The experiments reported therein were performed in quite another connection (6, 14) and although the smoothed curves of figure 5 represent a few discrepant points, the existence of the temperature function, in rough approximation to that indicated, would appear unquestionable. It may be mentioned that the growth temperature limits of Azotobacter are approximately 10°C. and 50°C.

It will be observed in figure 5 that below a certain temperature humic acid stimulation of growth velocity is negligible (about 20°C. in N_2, about 26° in urea-N). Above this temperature the stimulation ratio becomes finite and increases through some 10 to 12 degrees to attain a relatively constant value for possibly 5 degrees. The function takes the general form of an S-shaped curve between 0–40°C. The urea curve, as would be expected in view of the considerably greater velocity of growth in urea-N (see 6, 14, and the preceding section "Constant Temperature Conditions"), is considerably lower than in N_2. No respective data are available, but curves for NH_3-N, NO_3-N, and NO_2-N would undoubtedly lie intermediate, in view of their intermediate growth velocities (6, 14).

Table 8 brings out other interesting relations. The induction time is substantially independent of temperature, respiration rate at which stimulation commences, and the H. A.-velocity constant ratio. Likewise, the last-named function, although dependent upon temperature, is substantially independent of the respiration rate at which stimulation commences (12–90 cmm. O_2/cc./hr.) and, as just stated, the induction time, at least over the infra-optimal temperature range. The 2-hour induction values in N_2, above 33°C., may be significantly lower than values at lower temperatures.

The reason for sub-optimal temperatures yielding a humic acid velocity constant ratio function of g_0 the converse of that obtained in the case of all other investigated factors is not entirely clear. It is possible that temperature, as contrasted to all other factors, possesses some direct influence upon the permeability of humic acid, or upon its maintained concentration within the cell. It is very probable (6) that iron and temperature are the chief external factors regulating growth velocity under usual conditions and that increasing temperature increases not only growth velocity but also the relative need for iron to maintain the increased velocity. In the case of experiments made concerning the other factors, iron and temperature were substantially optimum and were not limiting. There was no reason to suppose that variation in other factors could greatly increase or alter the iron need, even when the growth velocities were increased, since as pointed out in the first and last paragraphs of the section "Constant Temperature Conditions," such increases in growth

TABLE 8

The influence of temperature upon the humic acid-velocity constant ratio in free and urea nitrogen (Warburg technique)

	SOURCE OF NITROGEN					
	N_2			Urea		
Factor............	Induction* time	Respiration rate at end† of induction (cmm. O_2/cc./hour)	H. A.-velocity constant ratio	Induction* time	Respiration rate at end† of induction (cmm. O_2/cc./hour)	H. A.-velocity constant ratio
Temperature °C.						
16	—	(9)	1.00	—	(10)	1.00
17	4	13	1.05	4	14	1.04
20	—	(18)	1.00	—	(19)	1.00
23.5	5	42	1.11	—	(35)	1.00
25	3	24	1.15	—	(24)	1.00
26.5	4	20	1.26	—	(22)	1.02
29.5	2	16	1.34	—	(28)	1.01
30	4	41	1.35	4	44	1.07
31	..	....	....	5	26	1.11
34	5	12	1.20	—	(14)	1.00
36	2	24	1.81	5	90	1.26
37	..	....	....	4	42	1.08
40	2	28	1.43	4	42	1.23

See (6, 14) for detailed description of the experiments. In brief, 1-day old cultures were invariably employed and growth measurements made for 7–8 hours.

* The time required for humic acid stimulation to become evident, i.e., for the humic acid ratio to become 1.03–1.05, and to continue to increase thereafter, or (again, i.e.) for the velocity constant in humic acid to definitely assume a new (and constant) value as represented by a new slope when "log. respiration rate" is plotted against time.

† i.e., the respiration rate when stimulation first commences. The values in parentheses in this column represent the respiration rates at an arbitrarily assumed time (fourth hour) in those cases where no stimulation occurs.

TABLE 9

The length of induction period given by natural humic acids in various experiments (Warburg technique)

INDUCTION* LENGTH	NITROGEN SOURCE (FIXED NITROGEN)	H. A.	g_0	$g_{H.A.}$	$\frac{g_{H.A.}}{g_0}$	RESPIRATION RATE AT BEGINNING OF STIMULATION	CULTURE† AGE	TEMPERATURE
hours	*p.p.m.*	*p.p.m.*				*cmm. O_2/cc./hour*	*days*	*°C.*
1	N_2	I-54	0.15	0.21	1.41	10	1	28
1	N_2	I-125	0.13	0.22	1.69	14	1	28
1	N_2	I-50	0.11	0.18	1.64	25	1(3)	28
2	N_2	II-50	0.07	0.12	1.67	86	2(4)	28
2	N_2	II-25	0.10	0.14	1.40	28	1	28
2	N_2	I-50	0.13	0.20	1.54	38	1	28
3	N_2	I-50	0.15	0.20	1.33	43	1	28
3	N_2	V-50	0.17	0.28	1.65	38	1 (2)	31
3	N_2	V-50	0.09	0.21	2.34	90	1	31
3	N_2	V-50	0.18	0.24	1.33	65	1	31
3	N_2	V-100	0.22	0.26	1.18	56	1	31
3	N_2	II-40	0.16	0.18	1.13	25	1	28
3	N_2	II-40	0.16	0.18	1.13	35	1	28
3	N_2	II-40	0.16	0.19	1.19	48	1	28
3	N_2	II-40	0.16	0.20	1.25	63	1	28
3	N_2	II-40	0.16	0.20	1.25	72	1	28
3	N_2	II-40	0.16	0.22	1.37	73	1	28
4	N_2	V-50	0.28	0.36	1.29	24	1	31
4	N_2	V-50	0.20	0.26	1.30	70	1	31
4	N_2	V-50	0.03	0.14	4.67	140	1	31
4	N_2	V-50	0.01	0.03	3.33	75	1	31
4	N_2	I-125	0.20	0.25	1.25	12	1	28
4	N_2	IV-25	0.12	0.19	1.58	43	1	28
4	N_2	IV-100	0.12	0.20	1.67	43	1	28
4	N_2	V-25	0.12	0.15	1.25	43	1	28
4	N_2	V-100	0.12	0.18	1.50	43	1	28
5	N_2	V-50	0.12	0.14	1.17	40	1	28
5	N_2	V-50	0.15	0.18	1.20	11	1	31
5	N_2	V-25	0.02	0.06	3.00	80	1	31
6	N_2	V-50	0.09	0.15	1.67	81	1	31
6	N_2	V-50	0.09	0.14	1.56	98	2	28
6	N_2	II-20	0.19	0.21	1.11	18	1	28
6	N_2	I-80	0.04	0.07	1.75	15	4 (6)	28
6	N_2	I-40	0.13	0.18	1.38	50	2 (3)	28
6	N_2	II-40	0.13	0.18	1.38	50	2 (3)	28
7	N_2	I-40	0.12	0.27	2.25	14	8 (27)	28
7	NH_3-N: 50	II-50	0.22	0.28	1.27	96	1	28
9	NO_3-N: 50	II-40	0.14	0.16	1.14	50	2 (4)	28
3‡	N_2	V-50	0.15	0.20	1.33	54	(1)	31
	NO_3-N: 50		0.22	0.25	1.14	65		
	NH_3-N: 50		0.23	0.26	1.13	70		
	Urea-N: 50		0.25	0.26	1.04	75		

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* See footnote, table 8.

† Subscript in parenthesis refers to initial degree of dilution, if any, with customary medium containing 1 per cent glucose.

‡ These bracketed experiments in free and fixed nitrogen performed simultaneously.

TABLE 9—*Concluded*

INDUCTION* LENGTH	NITROGEN SOURCE (FIXED NITROGEN)	H. A.	g_0	$g_{H.A.}$	$\frac{g_{H.A.}}{g_0}$	RESPIRATION RATE AT BEGINNING OF STIMULATION	CULTURE† AGE	TEMPERATURE
hours	*p.p.m.*	*p.p.m.*				*cmm. O_2/cc./hour*	*days*	*°C.*
3‡	N_2	II-50	0.10	0.17	1.70	12	1 (6)	28
	NO_3-N:50		0.10	0.22	1.38	13		
3‡	N_2	II-50	0.10	0.19	1.19	38	1	28
	NO_3-N:50		0.18	0.22	1.22	41		
8‡	N_2	I-125	0.19	0.23	1.21	20	1	28
	NH_3-N:50		0.26	0.37	1.42	25		
8‡	N_2	II-10	0.14	0.17	1.21	26	1	28
9	NH_3-N:50		0.23	0.30	1.30	66		

velocity tended toward a limiting maximum velocity determined chiefly by the other factors besides iron. In other words, the problem reduces to one of when iron is and is not the chief factor determining growth velocity and H. A.-velocity constant ratio.

THE INDUCTION PERIOD

Length

A certain length of time elapses after addition of humic acid to a culture before stimulation commences. This induction period varies, in general, from 1 to 10 hours, as shown in table 9. It is seen that 3 to 4 hours is the most frequent period, the distribution frequency being as follows: 1, 2, 3, 4, 5, 6, 7, 8, 9 hours, respectively, 3, 3, 19, 9, 3, 6, 2, 3, 2. Commencement of stimulation is defined as the time when the humic acid ratios commence to rise appreciably above 1, i.e., 1.03 to 1.05, and subsequent time values indicate, by increasing in value, that the values 1.03 to 1.05 were real, and did not represent experimental error. There has been occasional indication that induction values considerably greater than 9 hours occur sometimes in young, very highly diluted, rapidly growing cultures (see 8, tables 19 and 20). This is in some measure to be expected in view of the results shown in figure 4, which indicate that when a certain high velocity is reached, say g_o = about 0.2 – 0.25 (in the case of Azotobacter), substantially no humic acid stimulation is possible. The very prolonged onset of stimulation in such cases is therefore probably not induction, but simply *the absence of stimulation.*

Character of onset of stimulation

The onset of stimulation occurs rather sharply over a period of generally not more than one hour, as may be seen particularly in figure 6, but also in figures 7 and 8, and to some extent in tables 10 and 11. During induction $g_o = g_{H.A.}$, but at the onset of stimulation the new $g_{H.A.}$ is established suddenly; this is true, as seen in figure 6, independently of the length of induction, i.e., 1, 2, 3, 4, 4, 5, 5, 5, or 8 hours, in curves III, V, IX, VIII, VI, IV, VII, II, and I, respectively.

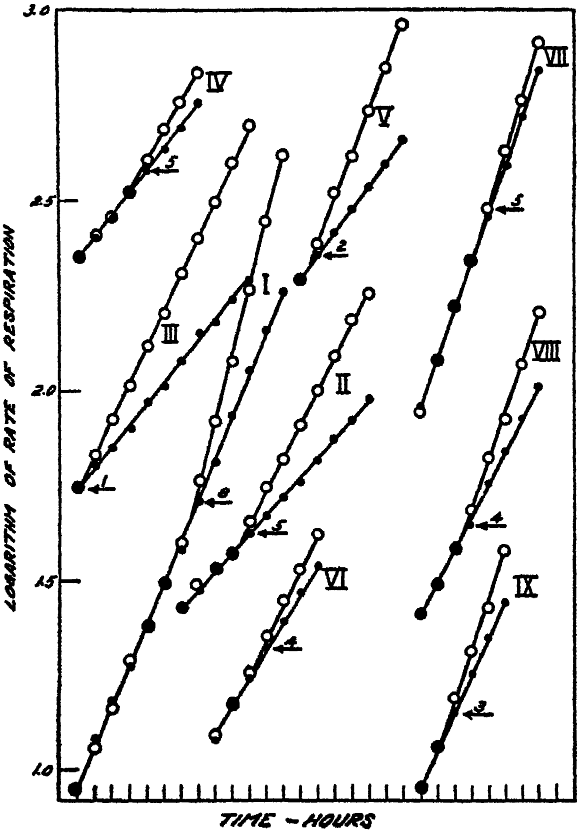

FIG. 6. THE SHARPLY DEFINED ONSET OF HUMIC ACID STIMULATION WITH DIFFERENT LENGTHS OF INDUCTION PERIOD, HUMIC ACIDS, TEMPERATURES, AND NITROGEN SOURCES (WARBURG TECHNIQUE)

Arrows indicate the approximate hour of definite commencement of stimulation and end of induction. In order to place all the nine stimulation curves in the figure the respiration rates have been calculated varyingly, per 1 cc., per 2 cc., etc.; this results in merely an ordinate shift of the entire curve. The cultures in the presence and absence of humic acid are indicated by circles and dots respectively. All cultures 1-day-old *A. vinelandii*, except No. IV, 2-day old.

Curve I: H. A.-I: 125 p.p.m.; 28°C.; per 2 cc; NH_3-N: 50 p.p.m.
Curve II: H. A.-II: 100 p.p.m.; 28°C.; per 1 cc.; NH_3-N: 50 p.p.m.
Curve III: H. A.-I: 125 p.p.m.; 28°C; per 4 cc.; N_2
Curve IV: H. A.-II: 40 p.p.m.; 28°C.; per 1 cc.; N_2
Curve V: H. A.-V: 60 p.p.m.; 36°C.; per 1 cc.; N_2
Curve VI: H. A.-V: 50 p.p.m.; 26.5°C.; per 1 cc.; N_2
Curve VII: H. A.-V: 50 p.p.m.; 31°C.; per 8 cc.; Urea-N: 100 p.p.m.
Curve VIII: H. A.-V: 50 p.p.m.; 31°C.; per 1 cc.; N_2
Curve IX: H. A.-V: 100 p.p.m.; 31°C.; per ½ cc.; N_2

TABLE 10

Comparison of induction periods of synthetic and natural humic acids (Warburg technique)

	EXPERIMENT 1		EXPERIMENT 2		EXPERIMENT 3	
	H. A.-ratio					
Humic acid, 50 p.p.m.	V (natural)	VII (synthetic)	V	VII	V	VII
hours						
Time, 1	1.00	1.00	1.00	1.00	1.00	1.00
2	1.00	1.00	1.01	1.02	1.00	1.00
3	1.00	1.00	1.14	1.02	1.00	1.00
4	1.02	0.99	1.23	1.11	1.01	1.00
5	0.99	1.02	1.42	1.15	1.01	1.00
6	1.01	1.01	1.52	1.22	1.02	1.04
7	1.06	1.04	1.57	1.26	0.99	1.06
8	1.07	1.05	1.89	1.41	1.02	1.07
9	1.13	1.11	2.59	1.99	1.16	1.13
10	....	....	2.95	2.15	1.17	1.22
12	1.17	1.18	....	....	....	....
13	1.17	1.20	....	....	....	....
14	1.20	1.24	....	....	....	....
16	....	....	3.56	3.06	1.28	1.36

In all experiments, 1-day-old cultures of *A. vinelandii*, pH 7.0 ± 0.1. Source of nitrogen: N_2. Initial respiration (cmm. O_2/cc./hr.). Experiments 1, 2, 3: 5, 15, 35.

TABLE 11

The influence of glucose concentration upon the length of induction period and humic acid ratio (Warburg technique)

	PER CENT GLUCOSE					
	0.12–0.15		1.12–1.15		H. A.-ratio	
50 p.p.m. H. A.-V		X		X	Low sugar	High sugar
Rate of respiration* (cmm. O_2/2 cc./hour)						
hours						
1	(12.4)	(8.6)	(11.6)	(8.0)	(–)	(–)
2	20.7	(20.7)	23.3	(23.3)	(1.00)	(1.00)
3	29.8	29.8	28.5	28.9	1.00	1.01
4	37.6	38.0	37.2	37.6	1.01	1.01
5	39.5	41.4	39.8	41.0	1.05	1.03
6	44.0	46.7	49.1	52.6	1.06	1.07
7½	47.9	50.0	55.6	61.6	1.04	1.11
8½	57.9	62.1	67.3	73.4	1.07	1.09
9½	64.5	70.2	86.7	99.0	1.09	1.10
12	86.0	96.3	120	142	1.12	1.18
13	102	116	155	184	1.14	1.19

Thirty-six-hour-old *A. vinelandii* diluted 5 times with customary medium. pH 6.8 ±0.1.

* Averages of duplicate determinations.

Figure 7 shows that the sharpness of onset of induction, as well as the length of induction, is substantially independent of concentration of humic acid, although perhaps not entirely. In this figure, as well as in figure 8, the slope of any curve at any moment is proportional to stimulation as measured by "$g_{H.A.} - g_o$," or the increase in velocity constant caused by humic acid, as is evident from the following formula $g_{stim} = g_{H.A.} - g_o = ln\ ((a + y_{H.A})/(a + y_o))/t = ln$ (H. A.-ratio)$/t$. If necessary, the humic acid velocity constant ratio may be obtained from the relation H. A.-V. C. R. = $(g_{stim} + g_o)/g_o$, g_o being given.

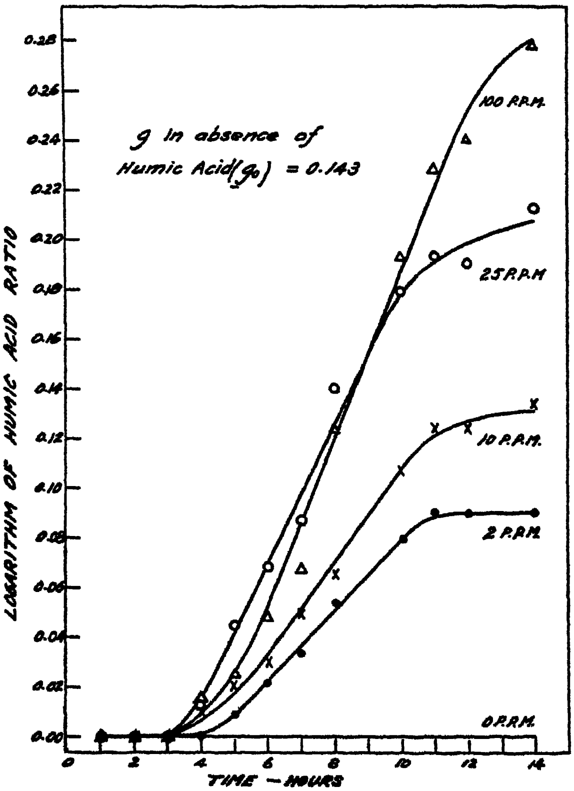

FIG. 7. HUMIC ACID STIMULATION AS A FUNCTION OF TIME AND CONCENTRATION OF HUMIC ACID (WARBURG TECHNIQUE)

One-day old *A. vinelandii.* 28°C. Final pH 7.0 ±0.1. H. A.-II employed. Source of nitrogen: N_2.

At all concentrations the induction periods in figure 7 last about 3 hours, and the new respective $g_{H.A.}$ values are established within 1 to 2 hours. It is possible that establishment in the case of 2 and 10 p.p.m. H. A. is somewhat longer than at 25 p.p.m. Triplication of cultures would be necessary to establish this minor point with certainty. It is definitely longer in the case of 100 p.p.m., even though the slope is slightly greater than at 25 p.p.m., because of the phenomenon, elsewhere referred to, that, at about 100 p.p.m. humic acid or greater, stimulation is at first inhibited slightly for secondary reasons, so that a combination of stimulation and temporary slight inhibition obtains.

The humic acid velocity constant ratio as a function of concentration, considered in figure 1, is well illustrated in figure 7 for the case of a single experiment. There is a gradual increase in $g_{H.A.}$ (i.e., $g_{H.A.} - g_o$) up to about 25–100 p.p.m. humic acid. The consumption of the active stimulating fraction of humic acid is also well illustrated in figure 7 by the ultimate decreases in slope to zero at 10–14 hours, occurring perhaps a little more sharply and sooner, the lower the concentration. The assumption of a horizontal or zero slope indicates that the active fraction of the humic acid has been entirely consumed and that the culture is then growing with the same velocity constant as the culture in the absence of humic acid.

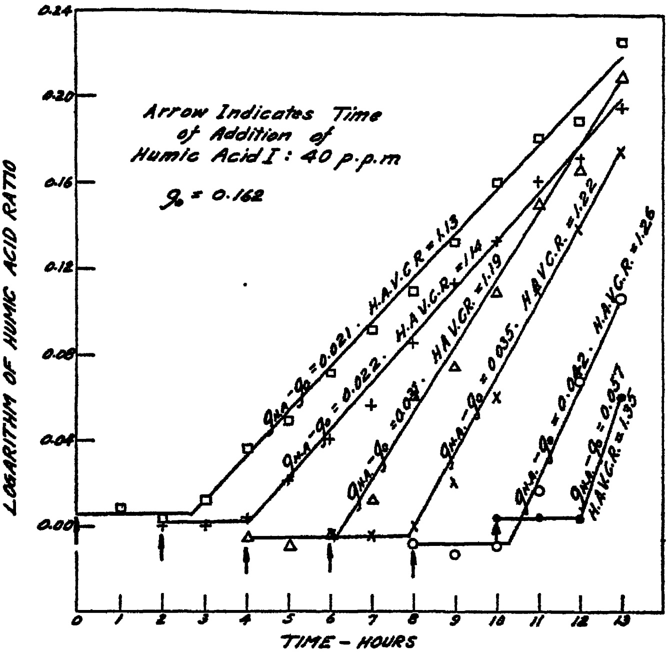

FIG. 8. THE INDUCTION PERIOD AS A FUNCTION OF MOMENT OF ADDITION (WARBURG TECHNIQUE)

One-day old *A. vinelandii.* 28°C. Final pH 6.9 ±0.1. 40 p.p.m. H. A.-II employed. Source of nitrogen: N_2. In this experiment a number of identical culture vessels were set up at $t = 0$, and humic acid added to each of two duplicate cultures at $t = 0, 2, 4, 6, 8$, and 10 hours, respectively.

The cause of induction

We have been unable to ascertain the chief factor determining length of induction period, but, as may be gathered particularly from table 9, and to some extent from tables 8, 10, 11, and figures 6, 7, 8, a great many factors have been investigated with negative results in every case. The length of induction period would appear to be substantially independent of temperature (table 8); source of nitrogen (tables 8, 9; fig. 6); sugar concentration (Table 11); humic acid preparation (Tables 9, 10; fig. 6); concentration of humic acid (table 9; figs. 6, 7); age of culture or degree of dilution of culture (table 9); respiration rate at onset of stimulation (tables 8, 9, 10; fig. 6); moment of addition (fig. 8); g_o (table 9; fig. 6); $g_{H.\,A.}$ (table 9; fig. 6); and H. A.-velocity constant ratio (table 9, fig. 6).

Plots of data in table 9 of induction period length against either g_o, $g_{H.\,A.}$, $g_{H.\,A.}/g_o$, or "respiration rate at beginning of stimulation" yield no detectable function; the points are scattered in an entirely random manner.

The experiment represented in figure 8 was performed in order to eliminate the possibility that the observed induction period occurred because of some property peculiar to *Warburg* technique experiments. According to this possibility cultures so grown might require a certain period of adjustment in the apparatus before being susceptible to stimulation. It is seen, however, that this possibility does not obtain. As the moment of addition of humic acid to the culture is varied from 0, 2, 4, 6, 8 and 10 hours the induction period remains approximately constant at 3 to 4 hours, and then, in every case, the new velocity constant of stimulation is established within an hour or two. It is of interest to note the slight increase of $g_{H.\,A.}$ and H. A.-V. C. R. with time or moment of addition.

In view of the aforementioned negative evidence with respect to so many factors it would seem probable that the existence of the induction period is determined by some purely physiological factor such as permeability or rate of entrance of the active stimulating fraction of humic acid into the cells, which is beyond the scope of this paper to investigate.[2]

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[2] Iwasaki (11), working in Meyerhof's laboratory, has also observed that at 28°C. a sharply defined induction period occurs which is relatively independent of the concentration of humic acid; that the action of humic acid is due to the consumption of some constituent, since the length of time over which humic acid stimulation continues increases with the concentration; and that, as we have shown elsewhere (2, 3, 4), (*a*) humic acid stimulates cultures grown in free or fixed nitrogen, (*b*) the stimulation is decreased as the oxygen pressure is decreased below 0.21 atmosphere, (*c*) the efficiency of growth increases as the rate of growth is stimulated by humic acid, (*d*) humic acid is heat-stabile at autoclaving temperatures. Iwasaki's work is therefore in substantial agreement with our work wherever the same phenomena have been investigated; it is less extensive, however, and subject to the disadvantage that velocity constants of growth, i.e., logarithmic functions of growth, were not employed. On this latter account, and because of greater experimental error, Iwasaki was unable to observe, as we have in figure 7, that stimulation as represented by an increase in the velocity constant of growth is a definite function of concentration of humic acid.

Induction in humic acid substitution compounds

Various natural humic acids present minor differences in induction length, and consequently the same differences should be expected between various synthetic iron-containing humic acids or between the latter and natural ones. Table 10 illustrates a few experiments comparing the synthetic and natural humic acids, VII and V, in which it is seen that roughly the same induction period obtains in both cases. In the light of all our work on this subject, it can be said that, in general, synthetic acids can be prepared to yield the same induction period, viz., a few hours, as the natural humic acids, but that sometimes a given synthetic humic acid will show a distinctly longer induction period.

As a result of gathering together a considerable number of pertinent experiments similar in import to those in table 9, it may be said that organic acid iron compounds of the type of ferric citrate show an induction period of generally 8 to 15 hours (very occasionally less, however, or even more), whereas with inorganic iron compounds the induction is generally 15 to 30 hours.[3]

The Influence of Humic Acid upon the Oxidation-Reduction Potential of the Medium

Studies carried out during the last decade, and in particular during the last year or two [Knight (12), Hewitt (10), and Allyn and Baldwin (1)] have indicated that the oxidation-reduction (O/R) potential of the medium may influence markedly the growth of bacteria, and *vice versa*. In view of the proved connection of humic acid stimulation with iron (8), and the relation of iron, even in small concentrations, to systems of interest in connection with O/R potentials, it is obviously pertinent to determine whether humic acid stimulation is related to any change which humic acid may impose upon the O/R potential of the medium.

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[3] It is not difficult to understand, on grounds of solubility alone, why inorganic iron should show much the greatest induction phase. In stating in the various experiments that inorganic iron of a certain concentration is added, no implication is intended that any considerable fraction remains in solution. Thus an addition of 2 p.p.m. Fe as $Fe_2(SO_4)_3$ would yield about 0.01 p.p.m. soluble Fe. The initial soluble iron content of customary medium containing 1 per cent glucose is considerably higher (about 0.035 to 0.057 p.p.m.). Since the minimum equilibrium concentration of iron of any type yielding optimum stimulation of growth is about 0.5 p.p.m., *inorganic iron must almost always be limiting, and will compare favorably with humic acid iron* (*etc.*) *only when the latter is supplied in similarly limiting concentrations.* This point cannot be too strongly emphasized. Upon this basis it is easy to understand the universal observation that the ash of humic acid, containing iron, is usually relatively inactive compared to the original sample of unashed humic acid. On the other hand, all humic acid and organic acid iron is soluble. Since the induction phase is still slightly greater in the latter case than in the former case it is evident that solubility is not the sole physiological factor governing differences in induction-time values between different iron-compound types. The fact that the iron in humic acid is partly in an adsorbed condition very probably explains its greater availability compared to iron bound to organic acids, which is in chiefly a non-ionic complex form. Finally, it is to be pointed out that although the simple organic acid and inorganic iron compounds show in general greater periods of induction than natural (or synthetic) humic acids, the fact that they show induction at all is in itself additional evidence in favor of the view that iron is the cause of humic acid stimulation, because of the qualitative similarity to the humic acids; since the simple iron compounds obviously possess secondary chemical differences they can be expected to possess secondary physiological differences.

We have employed the La Motte series of dyes: 1. o-chlorophenol indophenol, 2. phenol indophenol, 3. o-cresol indophenol, 4. 1-napthol-2-sulfonate indophenol, 5. methylene blue, 6. indigo tetrasulfonate, 7. indigo disulfonate, and 8. indigo monosulfonate, with respective E_0 values at pH 7.0 of 0.233, 0.229, 0.195, 0.119, 0.011, −0.046, −0.125, and −0.157. The cultures have always been adjusted initially to pH 7.0, by the appropriate and necessary addition of either mono- or di-potassium phosphate. The *Warburg* technique has been used for experiments conducted in an oxygen-free atmosphere, and both the *Warburg* and *Erlenmeyer* techniques for cultures maintained in air. In the anaerobic experiments care was taken to have removed the last traces of oxygen before adding the dye to the medium. In general the colorimetric dye comparison measurements were accurate to ± 5 per cent.

TABLE 12

The influence of hydrogen as compared to nitrogen upon the O/R potential under anaerobic conditions (Warburg technique)

	GAS					
	N_2	H_2	N_2	H_2	N_2	H_2
20 p.p.m. dye	2	2	5	5	7	7
Per cent reduction, 5 minutes	40	40	..	..	..	..
Per cent reduction, 18 minutes	..	..	50	50	..	..
Per cent reduction, 180 minutes	..	..	..	..	40	40

Three-day-old *A. vinelandii.* 31°C. Dyes tipped into cultures from side-cups of respiration vessels after last traces of oxygen removed as shown by zero respiration readings (KOH in the well). Experiments performed in duplicate.

Call suspensions of Azotobacter, in common with those of many other bacteria [Cannan, Cohen, and Clark (9)], are poorly poised with respect to the electromotively active material present at any moment. With cultures at least 1 day old, the percentage rates of reduction are relatively independent of concentration of dye up to 100–200–500 p.p.m. In air, a positive O/R potential of about +0.20 volt is assumed, as indicated by partial reduction (in general 50 per cent, see table 14) of dye 3, attained after one-half to several hours. The rate depends a good deal upon the concentration of dye employed and the extent of shaking employed to maintain equilibrium with the 21 per cent O_2 in the air which the cultures through respiration tend to lower. Dye 2 is reduced completely and dye 4 is not reduced at all. In N_2, a negative potential of about −0.15 v. is assumed after some hours of anaerobiosis (see table 13). Dyes 1, 2, 3 (at concentrations of 5–100 p.p.m.) are reduced 95 per cent in about 15 minutes, dye 4,95 per cent in about 30 minutes, dye 5, 95 per cent in 1 to 2 hours, and dye 7, 95 per cent in 6–12 hours. Reduction of dye 8 has never been observed to an extent greater than 5 ± 5 per cent. Upon admission of air to, say 90–95 per cent reduced dye 7 (in anaerobic

Azotobacter cultures), the oxidized form (0–10 per cent reduced) is assumed in a few seconds, and to other more positive dyes correspondingly longer times (1 to 15 minutes); with dyes 1 and 2 little or no reoxidation is obtained. In H_2 the same results are obtained as in nitrogen (see, for example, table 12). The experiments with hydrogen, exampled by table 12, are important in showing, even more delicately than hitherto (2), that Azotobacter is unable to consume hydrogen gas, i.e., hydrogen is completely inert, and that Azotobacter possesses no

TABLE 13

The influence of natural and synthetic humic acids upon the O/R potential of cultures of customary medium maintained in pure N_2 (Warburg technique)

Experiment 1:												
P.p.m. dye 7..	4	8	30	90	15	15	15	15	15	15	15	15
P.p.m. H. A...	–	–	–	–	–	–	V–10	V–150	VI–50	IX–50	X–50	XVIII–50
Per cent reduction, 5 hours.......	90	65	65	70	80	75	85	60	75	55	55	65
Experiment 2:												
P.p.m. dye 7..	10	10	40	40	100	100	200	200	(Dye 8 10)	..	...	...
40 p.p.m. H. A.-V.......	+	–	+	–	+	–	+	–	–	..	...	...
Per cent reduction, 11 hours.......	..	..	95	95	85	85	...	...	0–5	..	...	...
Per cent reduction, 17 hours.......	95	95	95	95	95	95	75	75	..	..	...	...
Per cent reduction, 21 hours.......	..	..	..	..	..	..	85	85	..	..	...	...
Experiment 3:												
P.p.m. dye 7..	20	20	20	20	20	20	20	20	5	5	100	100
P.p.m. H. A.-V..........	5	–	20	–	40	–	150	–	40	–	40	–
Per cent reduction, 17 hours.......	0	0	0	0	0	0	0	0	0	0	0	0
Experiment 4:												
50 p.p.m. dye.	3	3	3	4	4	4	...	..	..	..	...	...
10 p.p.m. dye.	..	..	..	..	...	...	5	5	5	..	...	...
P.p.m. H. A....	–	V	VI	–	V	VI	–	V	VI	..	...	...
Per cent reduction, 7 hours......	0	0	0	0	0	0	0	0	0	..	...	...

Experiment 1: 3-day-old *A. vinelandii*. 31°C.

Experiment 2: 2-day-old *A. vinelandii*. 31°C.

Experiment 3: Customary medium containing 1 per cent glucose (no Azotobacter). About 23°C.

Experiment 4: Customary medium containing 1 per cent glucose (no Azotobacter). 31°C.

Experiments performed in duplicate.

hydrogenase similar to that recently discovered by Stephenson and Strickland (16) capable of activating hydrogen gas to reduce oxygen gas, methylene blue, nitrate and fumarate, etc. The medium in the absence of Azotobacter ("customary" medium containing 1 per cent glucose) has no detectable effect on dye reduction either aerobic or anaerobic (see table 13), under the various experimental conditions employed. In short-time, aerobic, *Warburg* technique experiments, no beneficial effect of dye on growth (respiration rate increase) was observed, when 10 and 100 p.p.m. dye 2, 5 and 50 p.p.m. dye 5, and 5, 25, and 100 p.p.m. dye 7 were used; whereas in the same experiments, H. A.-V exerted a stimulating effect by the second hour and had established by the sixth hour a humic acid ratio of 1.5. O/R effects are substantially independent of the age of culture. In one experiment, in N_2, using 1, 3, 7, 10, 12, 13, and 38-day-old cultures, 75–90 per cent reduction of 20 p.p.m. of dye 7, at about 23°C., was obtained in 8 hours and remained thus for 30 hours. In another experiment, in air, using 1, 2, 5, 7, 14, 19, 20, and 44-day-old cultures, 20–90 per cent reduction of 50 p.p.m. of dye 3 at about 23°C. was obtained in ½ to 3 hours, and maintained thereafter indefinitely. The younger 1- and 2-day-old cultures required somewhat the longer reduction times, and in general, with very young cultures one must employ relatively smaller concentrations of dye to obtain equal reduction times as compared with cultures 2 days old or more; the O/R capacity factor is undoubtedly smaller in very young cultures. The effect of oxygen tension upon O/R potential of cultures is easily observed. In one particular experiment with 5-day-old *A. vinelandii* maintained in *Warburg* vessels at 100, 21, and 2 per cent O_2 (in N_2) the per cent reductions of 50 p.p.m. of dye 3 obtained after 3 hours were 20, 50, and 90 per cent, respectively (corresponding to a maximum difference of about 0.05 volt). It is highly probable that the unique oxygen pressure functions of growth, respiration, and growth efficiency (3) are in some degree related to O/R potentials established at different oxygen pressures.

TABLE 14

The influence of natural and synthetic humic acids upon the O/R potential of cultures or customary medium maintained in air (Erlenmeyer technique)

Experiment 1:								
20 p.p.m. dye	3	3	3	3	4	4	4	4
P.p.m. H. A.-V	0	20	50	100	0	20	50	100
Per cent reduction, 1 hour	40	40	40	40	0	0	0	0
Per cent reduction, 2 hours	40	40	40	40	0	0	0	0
Experiment 2:								
P.p.m. dye 3	50	50	150	150	.	..	..	...
50 p.p.m. H. A.-V	50	..	50	...	.	..	..	...
Per cent reduction, ½ hour	35	35	15	15	.	..	..	...
Per cent reduction, 1½ hour	35	35	15	15	.	..	..	...
Experiment 3:								
P.p.m. dye 3	50	50	50	50	.	..	..	...
50 p.p.m. H. A.	..	V	VI	X	.	..	..	...
Per cent reduction, 20 minutes	80	80	80	80	.	..	..	...
Experiment 4:								
P.p.m. dye 3	20	20	20	...	.	..	..	...
25 p.p.m. H. A.	..	V	X	...	.	..	..	...
Per cent reduction, 5 hours	50	50	50	...	.	..	..	...
Per cent reduction, 24 hours	50	50	50	...	.	..	..	...

Experiments 1, 2, 3, 4: 3, 3, 17, 5-day-old *A. vinelandii*. All experiments in duplicate except No. 3, in triplicate, temperature about 23°C.

As seen in tables 13 and 14, humic acid exerts no measurable effect upon the O/R potential of either cultures of *Azotobacter* or the customary medium maintained under either aerobic or anaerobic conditions. This is true for both natural humic acids (H. A.-V) and synthetic humic acids, independently of whether the latter are iron-rich (viz., H. A.-IX, X) or iron-poor (viz., H. A.-VI, XVIII). Moreover, the rates of dye reduction are likewise completely unaffected. Concentrations of humic acid varying from 4 to 150 p.p.m. have been employed, and the dyes have varied in concentration from 5 to 200 p.p.m. Although humic acid might exert an O/R potential of exceedingly small magnitude, possibly detectable by electrometric technique, it is certain from these results that, so far as growth of Azotobacter cultures is concerned, no significant influence is involved.

Does Humic Acid Act by Increasing Directly the Availability of the Known Constituents and Physical Properties of the Medium?

Tables 15 to 19 show that the humic acid ratio is on the whole independent of the nature of the inorganic medium in experiments of ordinary duration. The variations between the limits 1.4 and 4.5 are quite random, and can be assigned consistently to no particular inorganic causes. The independence is particularly striking, as shown in experiment 7, table 15, and in the case of the first two media employed in table 17; on the one hand, all the customarily added ions are present, and, on the other hand, the only other inorganic ion present is Na (except NO_3 and HCO_3, but these are present in both cases as a result of addition or of production in respiration). Tables 16 and 17 show particularly that the humic acid ratio is not significantly affected by either the presence or the absence of Ca, Mg, Fe, K, Na, Cl, PO_4, or SO_4, under the given conditions. Table 18 shows that the inoculum employed in tables 15, 16, and 17 can not have supplied sufficient of the customary inorganic salts to suffice for one generation or transfer, since after seven transfers the ratio has remained unchanged, within errors of experimental measurement. Table 19 eliminates, as far as possible, the likelihood of these customary salts being supplied in either the glucose or the bicarbonate employed and a number of other similar experiments, not reported, gave the same results also.

Table 20 shows that humic acid has no immediate and direct effect upon CO_2 production, glycolysis (the formation of lactic acid, or some other acids), glucose consumption, or O_2 consumption. The date of table 11, and also the data of table 19, as compared with that of table 18, in some measure offer additional confirmation with respect to glucose consumption, since the initial glucose concentration has been varied 10-fold. In several hundred experiments with the *Warburg* technique, where cultures have been maintained in the presence or absence of humic acid, it is ordinarily impossible to detect any appreciable increase (3 to 5 per cent) in respiration during the first hour after the addition of the humic acid, whereas, on the other hand, common stimulants or depressants of respiration, such as changes in O_2 tension, HCN, and in-different narcotics, almost invariably exert their complete effect within a few minutes.

The humic acid ratio does not depend upon the source of nitrogen available for growth, provided that the growth velocities in free and in the various forms of fixed nitrogen in the absence of humic acid are approximately equal [table 15; (8); tables 3A, 11A; (11); (2); (3); (4); (5)].

TABLE 15

Miscellaneous experiments on the magnitude of the humic acid ratio in the presence and absence of fixed nitrogen

EXPERIMENT NUMBER	MEDIUM	FINAL NET TURBIDITY (RELATIVE GROWTH)		H. A.-RATIO
		H. A.-I (25 p.p.m.)	No humic acid	
Experiment 1 (a):				
a. In N_2	Customary	450	100	4.5
b. In NO_3	Customary	982	295	3.3
Experiment 2 (a):				
a. In N_2	Customary	371	158	2.4
b. In NO_3	Customary	982	340	2.9
Experiment 3 (a):				
a. In N_2	Customary	522	143	3.6
b. In NO_3	Customary	886	384	2.3
Experiment 4 (a):				
a. In N_2	Customary	458	109	4.2
b. In NO_3	Customary	833	384	2.2
Experiment 5 (b):				
a. In N_2	Customary	99	30	3.3
Experiment 6 (c):				
a. In N_2	Customary	60	41	1.5
b. In NO_3	Customary	162	98	1.6
Experiment 7 (d):				
a. In NO_3	Customary	117	50	2.3
b. In NO_3	Bicarbonate	18	5	3.6
Experiment 8 (e):				
a. In NO_3	Customary	207	85.8	2.4
Experiment 9 (e):				
a. In NO_3	Bicarbonate	186	103	1.8
Experiment 10 (e):				
a. In NO_3	Customary	287	174	1.7

a, b, c, d, e, inoculated with 2 drops 3-day, 1 drop 2-day, 1 drop 4-day, 1 drop 2-day, 1 drop 2-day-old *A. vinelandii*; analyzed after 48, 41, 74, 52, 48 hours. The relative values are relative only within any given experiment, and not between different experiments.

In experiments 1, 3, and 4, specifically, and many other similar experiments, the humic acid ratio is lower in NO_3-N than in N_2-N under otherwise comparable conditions, undoubtedly because growth in NO_3-N in humic acid is approaching its ultimate value under the given conditions and the velocity of growth is decreasing relatively more rapidly.

It does not seem probable that humic acid exerts its effect by virtue of

TABLE 16

The magnitude of the humic acid ratio in bicarbonate medium with different salts added

	COLUMN 1		COLUMN 2		COLUMN 3		COLUMN 4		COLUMN 5		COLUMN 6		COLUMN 7		COLUMN 8	
Bicarbonate medium plus	Nothing		½ customary $CaSO_4$		$\frac{1}{10}$ customary $CaSO_4$		Customary $Fe_2(SO_4)_3$		$\frac{1}{10}$ customary $Fe_2(SO_4)_3$		Customary NaCl		Customary $MgSO_4$		Customary $\left.\begin{matrix}K_2HPO_4\\KH_2PO_4\end{matrix}\right\}$	
H. A.-I (25 p.p.m.)	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−
Final gross turbidity	102	32.2	65.3	31.3	79.5	22.9	104	70.0	94	39.4	91.0	26.7	110	69.8	115	67.0
	98	35.3	64.1	29.2	74.6	33.2	146	74.4	93	48.3	95.5	26.9	107	66.8	.....	51.2
Turbidity uninoculated control	5.8	2.3	12.7	10.9	4.9	2.5	36.7	27.4	10.4	7.4	5.8	2.9	3.8	2.3	3.9	2.3
Final average net turbidity = growth	94.2	31.5	52.3	19.3	72.2	25.5	88.3	44.8	83.1	36.5	87.5	23.9	105	66	111	56.8
H. A.-ratio	3.0	.....	2.7	.....	2.8	.....	2.0	.....	2.3	.....	3.7	.....	1.6	.....	1.9	.....

Inoculated with 1 drop of 2-day-old *A. vinelandii*, examined after 48 hours. Final pH in all flasks 7.5. Source of nitrogen: 50 p.p.m. NO_3-N. VI/21–23/30. Volumes of culture solutions 34 cc., not 25. cc., as ordinarily.

In this experiment the sugar was added after sterilization (as sterile glucose) rather than before sterilization, as usual in other experiments. Bicarbonate medium turns somewhat brown when sterilized with glucose, no doubt, because the pH is 7.5, which accelerates decomposition.

TABLE 17

The magnitude of the humic acid ratio in media of different inorganic compositions

	MEDIA																	
	Customary		$NaHCO_3$*		$KHCO_3$		$KHCO_3$ plus $CaSO_4$†		$KHCO_3$ plus $MgSO_4$†		$KHCO_3$ plus NaCl†		$KHCO_3$ plus $Fe_2(SO_4)_3$‡		$KHCO_3$ plus $CaSO_4$‡		$KHCO_3$ plus $Fe_2(SO_4)_3$†	
H. A.-I (25 p.p.m.)	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−
Final net turbidity = growth	115¶	72.5¶	31.2	21.2	47.6¶	20.3¶	20.2	10.4§	22.3	9.5§	39.5	21.5	46.5	22.2	32.1	16.0	Lost	19.1
H. A.-ratio‖	1.6	....	1.5	....	2.3	....	2.0	.....	2.3	....	1.8	....	2.1	....	2.0	....	—	....
Final pH	6.8	6.8	7.2	7.2	7.2	7.2	6.9	6.9	7.2	7.2	7.2	7.2	7.2	7.2	7.2	7.2	..	7.2

Inoculated with 1 drop of 2-day-old *A. vinelandii*. Analyzed after 72 hours. Source of nitrogen: 50 p.p.m. NO_3-N present in all cultures as KNO_3, except in $NaHCO_3$ cultures when present as $NaNO_3$. VI/4–7/30.

* NO_3 added as $NaNO_3$.

† At customary concentration.

‡ At one-tenth customary concentration.

§ The depressing effect of $CaSO_4$ and $MgSO_4$ in bicarbonate medium in both the presence and absence of humic acid is to be noted. This occasionally takes place in nitrate cultures (never in N_2 cultures).

¶ It is to be observed that the rate of growth in customary medium to that in bicarbonate medium is here 115/47.6 = 2.4 in the presence of humic acid, and 72.5/20.3 = 3.6 in the absence of humic acid.

deactivating metabolically produced toxic products, since it has already been found [(5), and much unpublished data] that, under our various experimental conditions at least, Azotobacter does not produce during its metabolism (for cultures up to 30 days old) any metabolic products which are toxic to either respiration, growth, or the chemical mechanism of nitrogen fixation.

Direct measurements with capillary tubes show that concentrations of 25 to 50 p.p.m. of natural humic acid change the viscosity and the surface tension of the customary medium by no more than a few per cent (i.e., within experimental error of measurement), if at all. Measurements of the influence of humic acid upon the potential difference (P. D.) between culture medium and organism have not been made, but a significant influence is reasonably precluded when it is considered that some preparations of humic acid are effective at concentrations as low as 1 to 3 p.p.m. It would seem probable, also, that

TABLE 18

The humic acid ratio as influenced by repeated transfer in bicarbonate medium

	MEDIUM			
	Bicarbonate		Customary	H. A.-ratio
	H. A.-I (25 p.p.m.)	No humic acid		
Solution designation	A	B	C	A/B
Transfer 1	100	33	400	. . .
Transfer 2	68.4 74.4 } 71.4	33.2 33.4 } 33.3	331 309 } 320	2.1
Transfer 3	84.2 77.2 } 80.8	38.9 39.7 } 39.3	610 518 } 564	2.1
Transfer 4	124	54	443	2.3
Transfer 5	101 89 } 95	51.4 50.6 } 51.0	307 339 } 323	1.9
Transfer 6	82 88.7 } 85.4	58.8 55.6 } 57.2	346 299 } 323	1.5
Transfer 7	103 90 } 97	49	450	2.0

Transfer 1 inoculated with 1 drop of 1-day-old *A. vinelandii*; transfer 2 inoculated with 1 drop of transfer 1, solution B, after 2 days' growth. Transfer 3 inoculated with 1 drop of transfer 2, solution B, after 2 days' growth, and so on—in each case the fresh transfer was made from the one of the bicarbonate medium-without-humic acid flasks just before analysis. Final pH: Solutions A and B, 7.5; solution C, 7.1. Source of nitrogen: 50 p.p.m. NO_3-N. III/–/30.

The growths in solution C are given to indicate the rate of growth in the bicarbonate medium as compared with that in the customary medium, and also to show that the organisms in solution B would, at any transfer, when inoculated in solution C, give approximately normal growth.

All growth values for every transfer are strictly relative to each other and to those in table 19.

in the experiments reported in tables 15–19, and others, the potential difference (P. D.) varied considerably because of the great differences in salt concentration.

Summarizing the data of this Section, we may point out that all the evidence indicates that humic acid does not improve any of the known physical or chemical properties of various widely different media. This conclusion substantiates the findings of the previous paper (8) that humic acid exerts its effect by *adding* something to media, namely, iron.

TABLE 19

The humic acid ratio in 10-fold diluted bicarbonate medium

	MEDIUM			
	10-fold diluted	Bicarbonate medium*	Customary	H. A.-ratio
	H. A.	No H A.	No H. A.	
Transfer 6†..............................	$\left.\begin{matrix}105\\108\end{matrix}\right\}$ 107	44.5	$\left.\begin{matrix}195\\203\end{matrix}\right\}$ 197‡	2.4

* The medium consisted of 0.1 the customary amounts of glucose and potassium bicarbonate, i.e., 0.1 per cent glucose, and 0.01 per cent (= 100 p.p.m.) $KHCO_3$. This was done in an attempt to eliminate possible impurities in these substances whose availability the humic acid might be increasing. Source of nitrogen: 50 p.p.m. NO_3N (recrystallized). III/–/30.

† The cultures were inoculated with the same inoculum used in inoculating transfer 6, table 18, i.e., from 5B. Analyzed after 48 hours. Growth values relative to those of table 22. Final pH: 7.3, except in customary medium, 7.0.

‡ This value is lower than those of table 18, because no doubt the glucose concentration has become limiting by the time a relative growth of 150–200 is reached.

TABLE 20

The influence of humic acid upon the production of weak acids during respiration

	COLUMN 1. KOH ABSENT (CMM. RESPIRED CO_2 DISSOLVED IN MEDIUM*)	COLUMN 2. KOH PRESENT (CMM. O_2 CONSUMED)
Experiment 1:†		
N_2....................	26.5, 25.4, 25.9, aver. 25.9	146, 149, 152, aver. 149
N_2 plus 50 p.p.m. H. A.-II..................	26.8, 25.4, 25.6, aver. 25.9	150, 151, 146, aver. 149
Experiment 2:‡		
N_2....................	14.4, 12.0, aver. 13.2	50.7, 50.3, aver. 50.5
N_2 plus 25 p.p.m. H. A.-II..................	13.5, 12.6, aver. 13.1	54.2, 45.2, aver. 49.5

* This value is directly proportional to the total respired CO_2.

† Two-day-old *A. vinelandii*. 2 cc. culture per vessel employed. Duration of experiment 60 minutes. Humic acid had been added to medium 30 minutes before readings taken. Gas phase: 21 per cent O_2 in N_2. Final pH, column 1, 6.6 ± 0.1; column 2, 6.8±0.1. VIII/-30/30.

‡ 1-day-old *A. vinelandii*. 1 cc. culture per vessel employed. Duration of experiment 12 hours. Gas phase: 1 per cent O_2 in N_2. Final pH, column 1, 6.6 ± 0.1; column 2, 7.0 ± 0.1. VIII/29/30.

SUMMARY

This paper presents various functional relationships obtaining when the growth of Azotobacter is stimulated by humic acid or iron compounds. Particular care is taken to distinguish those functions which depend upon time from those which do not. Frequent use is made of the velocity constant of growth, rather than the velocity of growth, in determining the stimulation functions.

Humic acid stimulation of Azotobacter growth involves an induction period of several hours, during which time no stimulation takes place. The induction period is substantially independent of temperature, source of nitrogen, glucose concentration, humic acid preparation, concentration of humic acid, age of culture, heaviness of culture, respiration rate, moment of addition, the growth velocity in the absence of humic acid, and the magnitude of the subsequent stimulation. Synthetic iron-containing humic acids may possess the same induction period as natural humic acids, but organic acid iron compounds and inorganic iron compounds possess definitely longer induction periods.

The induction period is sharply defined; when stimulation commences a new velocity constant of growth is assumed within about one hour, the value of the constant depending, among other factors, upon the concentration of humic acid.

Stimulation increase in growth velocity constant attains a maximum at about 50 p.p.m. humic acid, or about 0.5 p.p.m. iron, which is the same iron concentration as obtains with respect to the three other iron compound types: synthetic iron-containing humic acid, organic acid iron, and inorganic iron. As the active fraction of the stimulant is consumed, during growth, the initial concentration giving maximum growth may be increased to 5 to 15 p.p.m. iron, corresponding to about 500 to 1,500 p.p.m. natural humic acid.

At constant temperature all factors observed to cause increased velocity constants of growth caused both relative and absolute decreased stimulation, with respect to nitrogen source, pressure of nitrogen gas, concentration of fixed nitrogen, medium constitution, age of culture, heaviness of culture, glucose concentration, and oxygen pressure. When the velocity constant of growth is increased as a function of sub-optimal temperatures, the converse relationship obtains, i.e., stimulation is increased.

Natural and synthetic humic acids exert no significant influence upon the oxidation-reduction character of either cultures of Azotobacter or the culture medium in the absence of organisms, both with respect to rates of potential change and ultimate potentials assumed. The cause of humic acid stimulation is consequently independent of oxidation-reduction potential factors. In this connection, the oxidation-reduction character of Azotobacter cultures in the absence of humic acid has been described; under aerobic and anaerobic conditions, potentials of about +0.20 and −0.15 volts, respectively, are assumed. Oxidation-reduction dyes have not been observed to stimulate growth under conditions where humic acid stimulation takes place.

Humic acid does not act by increasing directly the availability of constituents normally added to, or present in, the medium, viz., glucose, oxygen, free or fixed nitrogen, carbon dioxide, Ca, Mg, Na, K, Fe, PO_4, Cl, SO_4, HCO_3; or by deactivating toxic metabolic products; or by affecting the surface tension, viscosity, or potential difference between culture medium and organism.

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BOOK REVIEWS

Literatursammlung aus dem Gesamtgebiet der Agrikulturchemie, vol. I. Bodenkunde. By H. NIKLAS, F. CZIBULKA AND A. HOCK. Edited by H. NIKLAS and A. HOCK. Verlag des Agrikulturchemischen Instituts Weihenstephan der Technischen Hochscule, München, Germany, 1931. Pp. xxxvi + 1008.

A century of research in the field of soil science has created an imposing mass of information in many languages, scattered through periodicals, monographs, dissertations, and reports. Most of the information of interest to students of soils and plants has been gathered and published since the beginning of the present century. He who would keep abreast of the times must be more or less familiar, not alone with the scientific literature in his chosen field, but in contiguous fields. To this end the authors have made a notable contribution.

There are eight sections in this volume. The first contains a reference to literature of a more or less general character. The second section—comprising nearly 200 pages—has to do with the formation of soils. The fields of geology, mineralogy, physics, chemistry, and climate are all represented in this group of references. In the third section—pages 217 to 408—there are given the titles of papers dealing specifically with soil chemistry. Similarly, section four—pages 411 to 590—is devoted to soil physics; section five—pages 590–767—, to the influence of external factors on soils; section six—pages 773 to 789—, to soil inventories; section seven—pages 801–911—to soils of different origin; and section eight—pages 915 to 939,—to the composition of soils, rocks, and minerals.

For the convenience of the reader who does not possess a ready command of German, the preface, introduction, and table of contents are given also in English. Throughout the body of the book, the general topics are given in both German and English. For instance, on page 69, the reader will find the general topic "Auswaschung—Leaching.": on page 130 is given "Tropische und subtropische Böden" and its English equivalent "Tropical and Subtropical Soils." In each instance the reference consists of the name of the author, or authors, and the title of the paper—the latter usually in German or English. Relatively few of the titles are given in the original where languages other than German or English are concerned.

The extent of the field covered is readily indicated by the approximately 20,000 titles appearing in this volume. As is noted by the authors, "The bibliographical collection—in the way we have accomplished it,—is not able to comprehend all corresponding work of the various countries. We neither intended nor were able to do so. On the other hand all competent work is mentioned that was discussed in one of the 18 central journals, respectively central organs previously referred to." They say further: "Our work offers most serviceable and extensive rudiments, and may in a large measure help to start in all other scientific fields in the same way as we did, the absolutely indispensable collection of literature, that hitherto from every point of view seems to be excessively disunited."

Literatursammlung aus dem Gesamtgebiet der Agriculturchemie, vol. II. Boden Untersuchung. By H. NIKLAS, F. CZIBULKA, AND A. HOCK. Edited by H. NIKLAS and A. HOCK. Verlag des Agrikulturchemischen Instituts Weihenstephan der Technischen Hockschule, München, Germany, 1931. Pp. xxviii + 199.

The organization of the references given in this volume is identical with that adopted for volume I of the series. The authors note that "The publishing of a special volume II 'Bibliographical List of the Entire Domain of Soil Analysis' was intended particularly on the one hand to put the importance of this subject,—that in the last decade has been thoroughly elaborated by scientists of various countries—to good account, and on the other hand to meet the wishes of all those, who work exclusively at the analysis of the soil, but on the other hand not in general at the science of soil. For in the course of the last years certainly the scientific field of 'Soil Analysis' has become an independent branch of science—complete in itself—that according to its whole condition can neither be completely arranged in the sphere of 'Soil Science' nor in the sphere of 'Plant Nutrition.'"

The field covered in this book on the methodology of soil investigations includes the major topics of sampling, chemical analysis, physical analysis, and microbiological analysis. Some space is devoted also to microscopic soil analysis, mineralogical soil analysis, and analysis of rocks. As in the first volume of the series, there is a subject index and an author index. Like the first volume, it will serve as an extremely useful—in fact, indispensable—reference book. It should be made readily available to every worker in the field of soil science and plant nutrition.

Man and Microbes. By STANHOPE BAYNE-JONES. The Williams & Wilkins Company, Baltimore, Md., and Associates in Cooperation with The Century of Progress Exposition, 1932. Pp. X + 128, figs. 16.

The commemoration of the 100th anniversary of the founding of the City of Chicago has paved the way for various enterprises of an educational character. One of these is The Century of Progress Exposition itself. In conjunction with the organization of the exposition, provision has been made for the publication of pamphlets, monographs, and books as a means of bringing about a better understanding of the contributions that science has made to the development of our present economic and social structure. As a popular treatise on the significance of microbes to human society, this book should find favor with the many busy folks who wish to keep in step with the progress of applied science.

The author tells us that "In this small book, I have tried to give the microbes their due, indicating their useful and essential activities as well as their devastating effects. The occasional spectacular havoc wrought by the germs of disease should not divert attention from the invaluable chemical services which hosts of microbes are quietly rendering man, animals and plants all the time."

The nine chapters of the book deal respectively with Microbes; Microbes and the Soil; Microbes in Air, Water and Sewage; Microbes in Industry; Microbes and Plants; Microbes and Insects; Microbes and Diseases of Animals Transmissible to Man; Microbes and Diseases of Man, and Microbes and Civilization.

The Waste Products of Agriculture. Their Utilization as Humus. By ALBERT HOWARD AND YESHWANT D. Wad. Humphrey Milford, Oxford University Press, London, New York, Toronto, Melbourne, Bombay, Calcutta, Madras, 1931. Pp. xiv + 167, plates 14, figs. 9.

The authors' understanding of life and conditions in the Far East have undoubtedly influenced their approach to the subject dealt with in this book. They say, in the preface: "One of the main features of crop production at the present day is waste. Except in the Far East, where the large indigenous population has to be fed from the produce of the country-side, little is being done to utilize completely the by-products of the farm in maintaining the fertility of the soil. The ever-growing supplies of agricultural produce, needed by industry and trade, have been provided either by taking up new land or by the purchase of artificial manures. Both these methods are uneconomic. The exploitation of virgin soil is a form of plunder. Any expenditure on fertilizers which can be avoided raises the cost of production, and therefore reduces the margin of profit. It needs no argument to urge that, in maintaining the fertility of the soil, the most careful attention should be paid to the utilization of the waste products of agriculture itself before any demands are made on capital—natural or acquired."

The six sections of the book consist of Introduction; Organic Matter and Soil Fertility; The Sources of Organic Matter; The Manufacture of Compost by the Indore Method; The Chief Factors in the Indore process; and Application to Other Areas. There are also four appendixes dealing with The Manurial Problem in India; Some Aspects of Soil Improvement in Relation to Crop Production; Nitrogen Transformation in the Decomposition of Natural Organic Materials at Different Stages of Growth; and An Experiment in the Management of Indian Labour.

To the student of the Far East this volume will prove both interesting and useful for it has to do with some of the basic factors in the life of the teeming millions of India. The fundamental principles which are followed in extracting human and animal food from ancient soils are an expression of age-long experience. Science is now beginning to touch the practice of the tropics.

The authors are to be commended for having made a distinct contribution to an important phase of soil fertility.

Arbeiten über Kalidüngung. By O. ECKSTEIN, A. JACOB, AND F. ALTEN. Verlagsgesellschaft für Ackerbau M. B. H., Berlin, 1931. Pp. 237, figs. 72.

The German Kalisyndikat established in 1929 an experiment station at Berlin-Lichterfelde. It was provided in the research program of the founders that the activities of the station deal with soil and plant nutrition problems not alone in Germany, but in various soil regions of temperate, subtropical, and tropical countries. This book constitutes a report on the present status of the research activities of the station.

Four general topics are dealt with by the authors; namely, objectives of an experiment station specifically dealing with potash research; a discussion of the physical plant and resources of the station; soil profiles and mapping of the land used by the experiment station at Berlin-Lichterfelde; and projects and results of the station. In the last division, we find the following: 1, Laboratory studies of soils with special consideration of tropical soils; 2, Comparative tests of different methods for the determination of soil potash readily assimilable by plants; 3, Studies of the content of potash in plants and its chemical combinations; 4, The influence of fertilization with potash on the composition of crop products; 5, Potash salts as protective agents of plants; 6, The physiological reaction of potash salts; 7, The fertilizer action of anions and other constituents of potash salts; 8, Tests of the fertilizer action of different materials containing potash and magnesia; 9, Studies on the compatability in mixtures of different commercial fertilizers; and 10, Investigations on the significance of potash for the animal organism.

The Microscopic Characters of Artificial Inorganic Solid Substances or Artificial Minerals, second edition. By ALEXANDER NEWTON WINCHELL. With a Chapter on The Universal Stage by Richard Conrad Emmons. John Wiley & Sons, Inc., New York, and Chapman & Hall, Ltd., London, 1931. Pp. xvii + 403, figs. 311.

This is the second edition of a book that has evidently made a useful place for itself among recent text and reference books. The scope of the field treated by the author is indicated by "Principles and Methods" of Part I and "Descriptions of Artificial Inorganic Solid Substances" of Part II. There are, aside from the introduction, eight chapters in Part I, dealing with Crystals; Apparatus and Preparation of Materials; Optic Properties of Isotropic Substances; Optic Properties of Uniaxial Crystals; Uniaxial Crystals in Parallel Polarized Light; Optic Properties of Biaxial Crystals; Biaxial Crystals in Parallel Polarized Light; and The Universal Stage. The eleven chapters of Part II include: Elements; Sulphides, Etc.; Halides and Cyanides; Cyanates and Thiocyanates; Oxides; Carbonates, Etc.; Borates, Etc.; Sulphates, Etc.; Phosphates, Etc.; Columbates, Tantalates, Titanates and Zirconates; and

Silicates. The third and last part of the book, entitled "Determinative Tables," includes Table I, Isotropic Artificial Solid Substances; Table II, Anisotropic Artificial Inorganic Solid Substances; and Index. The illustrations and general workmanship are in keeping with the excellence of the standards maintained by the publishers.

Report for 1930 of the Rothamsted Experimental Station, Harpenden, Herts, England. By SIR E. JOHN RUSSELL, Director. Gibbs & Bamforth, Ltd., St. Albans. Pp. 172.

The reader of this report will be gratified to find that the research staff of the Rothamsted Station is continuing to make substantial additions to our knowledge in the fields of soil and plant science. The report for 1930 contains information on the organization as well as on the activities of the station. The list of staff members, of temporary workers, and of the publications of the station supplement similar information found in earlier reports. The results of the studies reported for the year relate to field experiments with cereals and other crops; effects of farmyard manure; a new continuous experiment; rotation I; effect of weather conditions on fertilizer efficiency; grasslands; accuracy of field experiments; soil microorganisms; cultivation of the soil; utilization of results of agricultural experiments; composition of the soil—soil analysis; composition of crops; the plant in disease; the farm—geological survey; field experiments at outside centers; fungous diseases in crops at Rothamsted and Woburn; observations on insects attacking farm crops; Rothamsted farm report; and microbiological observations.

The following sections of the report include summaries of papers published in 1930 pertaining to scientific, technical, and other papers; Woburn Experimental Farm; yields of experimental plots, 1930; trustees and committee of management—Lawes Agricultural Trust; and society for extending the Rothamsted experiments.

Results of Vegetation and Laboratory Experiments. The Fifteenth Annual Report (Russian) of the Agricultural Chemical Experiment Station, Petrovsko-Razumovskoye, 1930. By D. N. PRIANISHNIKOV. Published by the Agricultural Chemical Experiment Station of the Timeryazev Academy, Petrovsko-Razumovskoye, U. S. S. R., 1930. Pp. xii + 544, figs. 145.

The present report includes some of the work of the station for the years 1927 and 1928. A short introduction and a list of staff members and collaborators are followed by a series of papers whose titles are given in the following. Each paper is followed by a short résumé in German. The list of authors and titles of these papers follows: S. N. Rosanow and E. K. Lukownikow—"Thermophosphate aus dem Chibiner Apatit-Nephelin Gestein"; W. M. Kletschkowsky—"Ueber die Konstanz und über die Verschiebung der Wirkungsfaktoren in der Ertragsformel von Mitscherlich"; A. G. Schestakow—"Ueber die Rolle des Kalziums bei der Assimilation von Eisen durch die

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